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The impacts of colloidal material on the fate and transport of 17 B-estradiol in three Iowa soils

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by

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To:

My wife and son for their support

by the Grace of God

and for His Glory

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Chapter 1 : General introduction

Regions with both confined animal feeding operations and extensive row-crop (e.g., corn and soybean) production face a unique set of environmental challenges, centered on manure in its dual role as animal waste and crop fertilizer. This is partly due to the common disconnect whereby crop and livestock production are separate operations: most livestock producers do not have as much land available as would be optimal for agronomic manure application. The result is more manure applied to less land, exceeding both the crop's ability to take up the nutrients and the soil's ability to immobilize the contaminants. Tile drains or a high water table then allow otherwise immobile contaminants to be transported to surface waters or groundwater.

"Emerging contaminants" are previously overlooked contaminants that are of concern even at very low concentrations, such as hormones, pesticides, and pharmaceuticals. Due to their steroidal structure and phenolic ring, estrogenic hormones are being used as models for understanding the behavior of other emerging contaminants. This thesis focuses on estrogenic hormones.

Estrogens have been detected in surface waters at concentrations considered to have a biological/ecological impact. While not all of the sources or pathways to surface water have been identified, possibilities include waste water treatment effluent and biosolids, run-off/erosion of manure- and biosolids-amended lands, and leaching through soil to karsts, shallow groundwater, or agricultural tile drains. This thesis focuses on this last scenario.

Tile drainage systems form a shortcut for water infiltrating into agricultural fields to reach surface waters. They lower the water table by providing a drain or outlet, one to two meters deep in the soil that empties into a nearby ditch or creek. This shortcut decreases the residence time that percolating water and chemical contaminants have in the soil before entering surface waters. Because soil is biologically more reactive than saturated zones and surface waters, reducing a contaminant's residence time in the soil allows greater quantities to escape degradation and dissipation and reach surface waters.

Previous research has shown that estrogens preferentially sorb to soil organic matter (SOM), and that they are rapidly degraded in aerobic conditions. Consequently we usually expect little or no leaching and transport of estrogens in soil. However, estrogens have still been found below the depths predicted by traditional solute transport models. Explanations for this require the influence of preferential flow paths, with or without mobile SOM or some other colloidal materials. The high sorption intensity of SOM indicates that, if colloids containing SOM were mobilized in the presence of macropores, colloids could potentially carry estrogens deeper into a soil profile, reaching shallow groundwater or surface waters via tile drains. Once the colloids arrived in surface waters, the estrogens could desorb (due to the lower solution concentration now surrounding them) and become biologically active. This could lead to skewed sex ratios in fish and amphibian populations, potentially destabilizing the ecosystem. There is also potential for as yet unknown low-level effects on mammals that drink the water.

The above concerns about aquatic ecosystems, drinking water, and estrogens in the environment inform the broader questions motivating this research: How might the presence of suspended colloidal material affect the fate and transport of estrogens in soil? And how

might these effects be modified by soil texture, soil and colloidal organic matter content, and soil structure?

THESIS ORGANIZATION

This thesis has the form of four papers to be submitted for publication in *Journal of Environmental Quality*. The paper titles are:

1. "Sorption of 17β-estradiol and estrone to soil materials and environmental colloids"
    - This paper is an investigation into the sorption intensity of 17β-estradiol and estrone with three Iowa soil materials, water dispersible colloids from each soil material, and water dispersible colloids from swine manure.
2. "Reduction of estrone to 17β-estradiol in the presence of swine manure colloids"
    - This paper is an investigation of the reactions of 17β-estradiol and estrone with swine manure material.
3. "Sorption and degradation kinetics of 17 β-estradiol and estrone with soil materials and environmental colloids"
    - This paper is an investigation of the sorption and degradation rates of 17β-estradiol and estrone with three Iowa soil materials and water dispersible colloids fractionated from each soil material.
4. "Impacts of environmental colloids on the transport of 17-β estradiol in intact soil cores"
    - This paper is an investigation into the enhancement of 17β-estradiol transport in soils due to the presence of suspended colloidal material.

Each paper includes an abstract, introduction, materials and methods, results and discussion, conclusions, references, tables, and figures. The papers are preceded by a general introduction, and are followed by a general conclusion with suggestions for future research.

Chapter 2 : Sorption of 17β-estradiol and estrone to soil and environmental colloids

A paper to be submitted to the Journal of Environmental Quality

Jacob R. Prater, Michael L. Thompson, and Robert Horton

ABSTRACT

Estrogenic compounds occur in land-applied animal manure and pose a biological risk if they enter aquatic ecosystems through agricultural drainage or shallow groundwater. Therefore, it is important to understand the fate of estrogens in soil systems. Estrogens are nonpolar and strongly adsorbed by soil organic matter, limiting their miscible transport, but presenting another possible transport vector. The movement of colloids that are rich in organic matter may facilitate the transport of estrogens through soil and ultimately to surface water. As a first step in considering colloid mediated transport mechanisms, the objective of this study was to determine the intensity of adsorption of 17β-estradiol (E2) and estrone (E1) to three Iowa soils, to colloidal components of those soils, and colloidal components of swine manure. By using batch incubations, adsorption isotherms were measured for E2 and E1 binding to Clarion, Hanlon, and Zook soil materials, to the derived water-dispersible soil colloids, and to colloidal material fractionated from swine manure. Following incubation (24 h), solution concentrations of E2 and E1 were measured by using solid phase extraction to clean and concentrate the sample before final analysis by high pressure liquid chromatography. The soils selected ranged from 70 to 400 g kg^{-1} in clay content, 7 to 30 g kg^{-1} organic carbon, 12 to 60 cmol kg^{-1} CEC, and 7 to 11 in C/N ratio. Colloidal materials

ranged from 38 to 346 g kg^{-1} organic carbon. Measured partition coefficients (K_d values) ranged from 15 L kg^{-1} to 34 L kg^{-1} for the soil materials. The log K_{oc} values (organic carbon normalized K_d) for the soils were similar to values reported by others and ranged from 2.94 to 3.32. K_d values for colloidal material ranged from 72 L kg^{-1} to 213 L kg^{-1}. The log K_{oc} values for colloidal materials ranged from 2.79 to 3.33. The large K_d values for colloidal materials indicate a possibility for mobilized colloidal materials to carry these two estrogens through soil.

INTRODUCTION

Livestock such as swine as well as undomesticated animals excrete estrogenic hormones in the urine and feces. In the environment, the two most important and biologically potent of these estrogens are E2 and E1. There has been growing interest regarding the presence of estrogenic hormones from livestock production and human contraceptive use in aquatic ecosystems (Oberdorster et al., 2000; Corcoran et al., 2010) and in soil fauna and microbial systems (Markman et al., 2007; Chun et al., 2006). This interest has led to concerns about the environmental impacts of the use of hormones, livestock manure and human waste, and other endocrine disrupting chemicals (EDCs). Such EDCs can have biological effects on certain aquatic organisms at very low concentrations. For example, E2 levels of 1 ng L^{-1} or less have been found to have an effect on certain fish species, causing males to produce vitellogenin, a protein associated with egg production (Purdom et al., 1994; Hansen et al., 1998). Additionally, these chemicals have been shown to occur in waste water effluent and biosolids after escaping degradation or removal during treatment (Aga, 2008). EDCs are present in untreated, land-applied manure obtained from

concentrated animal-feeding operations (CAFOs) (Aga, 2008; Lange et al., 2002). Thus, understanding their fate in soil and water is paramount for maintaining water quality and healthy aquatic ecosystems.

Recent studies have shown that estrogen transport could be dominated by preferential flow and chemical and physical non-equilibrium conditions (Sangsupan et al., 2006; Steiner et al., 2010; Kjaer et al., 2007). Some studies have pointed to the possibility of enhanced estrogen transport by colloids (Steiner et al., 2010; Arnon et al., 2008; Casey et al., 2008).

The reactions of estrogens with soil (sorption, transformation, and degradation) have been observed by many researchers, but the conclusions have not always been the same. Because soils vary from region to region, chemical and physical interactions with estrogen are nuanced by soil properties. Different soils have yielded different shapes for sorption isotherms (linear and Freundlich), sorption reactions, and degradation reactions. Perhaps the reason for these variations is in the type of organic matter present, although numerous studies have pointed to roughly similar log K_{oc} values (an organic carbon normalized partitioning coefficient (K_d)) for E1 and E2 (Lee et al. 2003; Casey et al. 2003; Hildebrand et al. 2006). The focus of this paper is to investigate the interactions of organic carbon content, texture, and mineralogy on estrogen sorption intensity in three Iowa soils, their colloids, and swine manure colloids.

The role of colloids in inhibiting estrogen degradation has been noted by wastewater treatment engineers. They discovered that the amount of colloidal organic material in wastewater effluent affected its estrogenic activity, and thus, that the capture of colloidal material is a requisite step for reducing the estrogenic activity of wastewater effluent (Holbrook et al., 2003). This is noteworthy because, despite treatment (primarily of the

liquid phase), estrogenic activity persisted. In soils that receive applications of manure that is not treated, two modes of transport exist, miscible transport and colloidal transport. Because estrogens can be degraded and sorbed by soil materials, it remains that perhaps in ideal soil conditions for crop production (high organic matter and good drainage) the dominant transport mechanism may be colloid facilitated transport of these compounds. The reason for this is two-fold: sorption hysteresis can lead to slower desorption from the colloidal material, and the colloids may protect estrogens from some forms of degradation.

Reported log K_{oc} values for soil materials range from 2.3 to 4.2 for E2 and from 2.5 to 4.1 for E1 (Lee et al., 2003; Ying et al., 2003; Sarma et al., 2008; Sangsupan et al., 2006; Casey et al., 2005; Xie et al., 2008) while the reported values for river colloids are in the higher portion of the range of soils, 3.95 for E2 and 4.18 for E1 (Liu et al., 2005). Wastewater colloids have been reported to have similar values to river colloids ranging from 4.08 to 4.68 for E2, (Holbrook et al., 2003). Parameters describing sorption of estrogen compounds to fractionated soil colloidal material have not been reported, and only one other paper has reported on estrogen interaction with swine manure particulates (Amin et al., 2011). These researchers reported log K_{oc} values for the smallest size fraction of swine slurry separates (colloidal size) of 3.02 L kg^{-1} (Amin et al., 2012). The objective of this research was to determine the intensity of E2 and E1 sorption to three typical Iowa soils, to their associated colloids, and to swine manure colloids, highlighting any properties that might show linkage to sorption intensity with implications for the ability of colloidal materials to transport E2 and E1.

MATERIALS AND METHODS

Three annually cultivated Iowa soils, representing a spectrum of particle size distribution and organic matter content (soil series: Hanlon, Clarion, and Zook soils) were sampled in the top 10 cm at each of three sites near Ames, IA. Each soil sample was dried and ground to pass through a 2-mm sieve. Classification information for these soils is found in Table 2-1. The Clarion soil sample is from the Iowa State Curtiss Research Farm and is characterized as a well-drained upland soil formed in till under native prairie vegetation. The Hanlon soil sample is from the Iowa State Hinds Research Farm and is characterized as well drained, having formed in loamy alluvium under native prairie vegetation on natural levees along streams. The Zook soil sample is from the Ames Municipal Water Pollution Control Facility and is characterized as a poorly drained soil formed in silty and clayey alluvium and usually occurring in bottom lands. These three samples provide a particle size distribution from fine sandy loam to clay loam along with a broad spectrum of soil structural expression.

The soil colloidal material was dispersed by gentle agitation in distilled water using a rotary shaker at 15 rpm for 12 hours with a 1:8 soil to solution ratio (Kjaergaard et al., 1995). The resulting suspension was then fractionated using Stokes' Law (particle density, $\rho_s = 2.65$ g cm^{-3}) sedimentation for particles less than 1-μm (equivalent spherical diameter). The colloidal suspensions were then concentrated using auto irrigation pots (an automatic irrigation system adapted to be a porous ceramic filter apparatus) and subsequently freeze dried for later use.

Swine manure was collected from the flushing of a farrowing house at an Iowa State University research farm. The manure colloids were also fractionated using Stokes' Law sedimentation for particles less than 1-μm (assuming equivalent spherical diameter and ρ_s = 1.4 g cm^{-3}). Manure colloids were then concentrated onto 0.45-μm nylon filters and freeze dried for later use. Manure colloids were concentrated on nylon filters instead of auto irrigation pots in order to conserve the smaller amount of material and prevent the fouling of equipment.

After collection and fractionation, the soil and colloidal materials were analyzed for physical and chemical properties including particle size distribution using the pipette method (Gee and Or, 2002), soil organic carbon content by total combustion (Allison et al., 1965), pH (Peech, 1965), and cation exchange capacity using sodium saturation and ammonium acetate displacement (Chapman, 1965). A second measure of CEC was done without the removal of organic matter using the same method as the CEC measurement. The mineralogy of the colloid samples was determined using x-ray diffraction, with the samples being treated with H_2O_2 to remove organic matter, saturated with Mg, plated onto ceramic tiles, saturated with glycerol, analyzed using CuKα x-ray diffraction, and interpreted using the guidelines outlined by Whittig and Allardice (1986). An example of the x-ray patterns can be found in Fig. 2-1. Soil material physical and chemical properties are located in Table 2-2. Colloid properties are located in Table 2-3.

E1 and E2 were obtained from Sigma Aldrich (98% pure). Table 2-4 shows chemical structure (exhibiting a steroidal ring structure including a phenolic ring and few ionizable functional groups indicating their hydrophobicity) and properties of E2 and E1. The aqueous solubility of E2 and E1 are affected by both pH and ionic strength. Aqueous solubility

increases significantly at the pH where the compounds are ionized ($pK_a \sim pH$). Ionic strength has the effect of possibly coagulating these compounds in a 'salting out' effect thus, decreasing their solubilities at higher ionic strengths (Shareef et al., 2006). For these reasons a background electrolyte concentration of 5 mM $CaCl_2$ was maintained and solution pH was controlled by the soil samples (ranging from 5.5 to 6.5), but was well below the pK_a values of E2 and E1. Estrogen stock solutions were prepared using methanol as a co-solvent (less than 1% of total volume) followed by dilution in 0.005 M $CaCl_2$. The effect of methanol as a co-solvent in limited quantities on sorption has been shown to be negligible (Bouchard, 2003).

Equilibrium batch studies were conducted to measure the sorption intensity of each of the soil and colloidal materials for E2 and E1. These batch studies were carried out in 50-mL glass centrifuge tubes with Teflon-lined caps using dried and ground soil or colloidal material. Solid-to-solution ratios were chosen according to an estimated sorption intensity based on measured soil organic carbon (SOC) content. This was done to ensure that between 20% and 80% of the spiked estrogen (spikes of 0.05 – 2 mg L^{-1}) would remain in solution for efficient and accurate measurement. Solid-to-solution ratios were 1:100 for colloidal materials, 1:20 for Hanlon soil material, 1:40 for Clarion soil material, and 1:60 for Zook soil material. Significant effects of varying the solid-to-solution ratios on measured K_d values seemed unlikely given the reasonably consistent log K_{oc} values previously reported from sorption experiments with soils at different solid-solution ratios (Lee et al., 2003; Ying et al., 2003; Sarma et al., 2008; Sangsupan et al., 2006; Casey et al., 2005; Xie et al., 2008). Immediately before the sorption experiments, the soil and colloid samples were autoclaved (134^{o}C for 60 min) to inhibit microbial activity. After autoclaving, the samples were

equilibrated for 24 h on a reciprocating shaker. Following equilibration, each sample was centrifuged at 800 *g* for 16 min to separate the liquid and solid. An aliquot of the supernatant was then taken and adjusted to pH ~ 4 for analysis. Batch studies with soil were replicated 3 times and colloids were done singularly due to a short supply of the material. Solution and sorbed concentrations (determined by difference) were then plotted and fitted to linear isotherms with slopes denoted as partitioning coefficients (K_d). K_d values and log K_{oc} values (log of organic carbon normalized K_d values) were used to make comparisons between soils and colloids. Calculations of Kd values are found in Eq. 1, E_s (mg kg^{-1}) being the estrogen (E2 or E1) on the solid phase and E_{aq} (mg L^{-1}) being the estrogen in solution.

$$K_d = \frac{E_s}{E_{aq}} \qquad [1]$$

Concentrations of E2 and E1 in solution were quantified using high-pressure liquid chromatography (HPLC) (Beckman-Coulter HPLC, auto-sampler, and diode array detector, model #118, 507, and 168 respectively). Samples were prepared using a pre-concentration and clean-up solid phase extraction (SPE) with a C-18 substrate (LiChrolut EN) (Xie et al., 2008). Samples were then evaporated under a gentle N_2 gas stream at 40^{o}C and reconstituted in methanol. Instrument parameters for HPLC were similar to those used by Lee et al. (2003): Sample loop, 50 µL, flow rate, 1.5 mL min^{-1}; mobile phase, 45:55 acetonitrile: H_20 (pH ~4); column, C-18 reverse phase (Alltima); UV detector, λ = 202 nm. The method detection limit was 2.5 µg L^{-1} in a 30 mL sample (defined as 3 times the background signal representing a repeatable measurable peak) and the limit of quantitation was 6 µg L^{-1} in a 30 mL sample (defined as the lowest standard used). HPLC analysis was deemed more efficient

for our goals of measuring sorption properties compared to slower costlier methods with lower detection limits that are used to detect concentrations in purely aqueous systems.

RESULTS AND DISCUSSION

Water-dispersible colloids (WDC) had greater soil organic carbon (SOC) contents than the corresponding soil materials. Hanlon WDCs had 5 times more SOC than Hanlon soil materials, Clarion WDCs had 3 times more SOC than Clarion soil materials, and Zook WDCs had 1.3 times more SOC than Zook soil materials (Table 2-2 and 2-3). X-ray diffraction analysis showed a very large peak for smectite indicating that the clay fraction of all three of these soil colloidal materials were dominated by smectite (Fig. 2-1). CEC without OM removal for the soil materials were 20.1 cmol kg^{-1} for Clarion, 12.3 cmol kg^{-1} for Hanlon and 58.9 cmol kg^{-1} for Zook (Table 2-2). Particle size analysis indicated clay contents of 176 g kg^{-1} for Clarion, 72 g kg^{-1} for Hanlon and 400 g kg^{-1} for Zook (Table 2-2).

In all cases, WDCs had greater sorption intensities as indicated by the greater isotherm slopes and K_d values than corresponding soil materials (Fig. 2-2 and Table 2-5). Soil organic carbon content is a good predictor of sorption intensity for these three Iowa soils and their WDCs as evidenced by log K_{oc} values being very close to 3 for all materials (Table 2-5). Not all organic matter is equal with respect to sorption of these estrogens. With two alluvial soils (Hanlon and Zook) with similar log K_{oc} values and one till-derived soil (Clarion), there is something different (perhaps different quantities of fats, waxes, and aromatic carbon forms) about organic carbon forms in these soils as evidenced by the range of log K_{oc} values for both E2 and E1 (2.94 to 3.32 and 2.99 to 3.32 respectively). This range of log K_{oc} values corresponds to a 1200 and 1100 L kg^{-1} range in K_{oc} values for E2 and E1,

respectively. It is possible that Clarion, the upland soil, contains more fresh or undecomposed organic material being drier (upland landscape position) and that Zook also contains more fresh or non-pedogenic organic material having received bio-solids applications in the past both leading to slightly lower log K_{oc} values than Hanlon. This difference could mean that Hanlon contains more non-polar forms of SOC (fats, waxes, and aromatics) that might be associated with sorption of non-polar compounds like E2 and E1. However, these determinations are difficult to make without a more extensive data set of sorption intensity and organic matter characterization. An extensive characterization of organic matter in these soils could be used to test whether or not certain forms of organic carbon have greater affinity for, or sorption potential of E2 and E1.

Water-dispersible colloids from individual soils had higher K_d values than the corresponding soil materials (Table 2-5). This was particularly true in sandy, low-organic carbon Hanlon materials. The concentration of organic carbon (OC) in Hanlon colloids was 5 times more than in the bulk soil (Table 2-3). Average log K_{oc} values for soil and WDCs (3.17 for E2 and 3.23 for E1) displayed standard deviations of 0.16 and 0.18 for E2 and E1, respectively. This puts the log K_{oc} of manure colloids (2.79 for E2 and 2.81 for E1) 2.3 standard deviations from the mean of the soil and WDCs, an indication that the materials are distinctly different in terms of their interaction with E2 and E1. It is possible that the difference between the organic matter in soil and soil colloids and in swine manure colloids is the presence of less decomposed organic material. With the exception of Hanlon and E2, all log K_{oc} values were higher for WDCs than the corresponding soil materials. The greater log K_{oc} values for WDCs is consistent with the idea that the colloidal fraction of organic carbon is more degraded or more transformed pedogenically, thus, containing more

recalcitrant organic matter with more hydrophobic components like aliphatic chains and aromatic rings. If it is the case that there is a larger proportion of recalcitrant organic carbon forms in these materials then they would likely have higher log K_{oc} values due to having larger pools of non-polar organic matter. However, the greater log K_{oc} values for WDCs could also be an effect of higher surface area per unit mass on smaller particles. Differences in soil K_{oc} values for E2 and E1 by ecoregion (brown chernozems differing from other soils tested) have been noted in Alberta, Canada. Caron et al. (2010) also hypothesized that soils with more recalcitrant or more highly degraded organic matter may exhibit higher K_{oc} values. This is consistent with the mode of sorption being hydrophobic partitioning (as these more recalcitrant forms of SOC are more non-polar) which has been suggested by Lee et al. (2003) as the dominant sorption mechanism for these two estrogens to soils.

Conclusions about correlations between K_d and clay content, fine clay content, and CEC, with and without OM removal (not shown) are difficult to draw from this data set, but they may indicate that there is some sorption activity associated with clay minerals due to a weak correlation between K_d and clay and fine clay content, and CEC. Casey et al. (2003) also noted the relationship of K_d to clay content, pointing to smectite and CEC as additional potential sorption predictors alongside organic carbon content. Van Emmerik et al. (2003) noted that there was some affinity of smectite for E2 and suggested that E2 could be intercalated into the interlayer spaces of Ca- and K-saturated montmorillonite. Bonin and Simpson (2007) also noted that expanding clay minerals had a role in sorption of E2 and E1 to soils alongside organic carbon, but with a smaller effect. Bonin and Simpson (2007) suggested that the effect of mineral sorption sites with a different mechanism of sorption than partitioning were negligible when increasing soil organic matter was present. This could

explain some of the discrepancy between the log K_{oc} values of Hanlon, Clarion, and Zook with the low SOC soil Hanlon displaying a larger influence from mineral based sorption due to its lower organic carbon content and the other two soils exhibiting more of the effect of their larger organic carbon content and thus a muted mineral sorption effect.

The interaction of swine manure WDCs with E2 and E1 were different from those of the soil materials and soil WDCs as indicated previously by their large deviation (2.3 standard deviations) in log K_{oc} from the mean log K_{oc} of the other materials. Sorption isotherms are presented in Fig. 2-3. Swine manure colloids contained little if any mineral material and almost entirely consisted of microorganisms and partially digested organic matter. The chemical composition of the manure organic material was entirely different from the soil and soil colloids, with a much smaller C:N ratio than the soil derived materials (suggesting that the swine manure colloids were relatively protein rich) (Table 2-2 and 2-3). The difference in the interaction with estrogens is reflected by the lower log K_{oc} values for swine manure WDCs (Table 2-5). Consistent with the discussion above, lower log Koc values for the swine manure colloids most likely reflect the presence of hydrophilic compounds such as cellulose and protein residues.

CONCLUSIONS

Soils and colloids with large organic carbon concentrations have a strong affinity for sorbing estrogens. The K_d and log K_{oc} values for soils from this study are similar to other published data, but the information on colloidal material fills a gap in current knowledge. First, the soil organic matter is strongly associated with the colloidal size fraction, so colloid-estrogen interactions are reflective of the whole soil of origin, as evidenced by the similar log

K_{oc} values. Second, the colloids have much greater sorption intensity (indicated by the K_d values) than their soils of origin. Both of these findings, similar log K_{oc} values and higher K_d values for colloids than whole soils occur because most of the organic carbon in the soils associated with the colloidal size fractions. Variations in the log K_{oc} values among the soils may be related to differential decomposition due to soil formation processes resulting in a varying degree of hydrophobicity in the organic carbon present. Additional investigation into the forms of organic carbon as a result of soil formation as well as measurement of organic matter properties (nuclear magnetic resonance spectroscopy, fourier transform infrared spectroscopy, and thermal analysis) may reveal some of the reasons for differences in sorption behavior for soils and these compounds.

Beyond the differences between these materials, the high sorption intensities of the colloidal materials indicate a potential environmental impact. If colloids were mobilized and transport pathways present, colloids could potentially carry estrogens through a soil profile and impact shallow groundwater or enter surface waters via tile drainage systems. Further, colloid facilitated transport could be a greater contributor to estrogen transport than simple miscible transport due to the smaller, but still substantive, K_d values of the soil materials, which coupled with the greater mass of soil versus colloidal material, indicate that there would be a high degree of estrogen retention, via sorption, in soil and thus retardation of miscible estrogen transport in soil.

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Table 2-1. Soil taxonomic description and characteristics.

Soil	Hanlon	Clarion	Zook
Classification	Coarse-loamy, mixed, superactive, mesic Cumulic Hapludoll	Fine-loamy, mixed, superactive, mesic, Typic Hapludoll	Fine, smectitic, mesic Cumulic Vertic Endoaquoll
Parent material	Alluvium	Till	Colluvium/Alluvium
Surface horizon texture	Fine sandy loam	Loam	Silty clay loam
Landscape position	Floodplains	Convex uplands	Floodplains
Slope	0 – 2%	2 – 5%	0 – 2%
Drainage class	Moderately well drained	Well drained	Poorly drained
Organic matter in surface horizon	1 – 3%	3 – 4%	5 – 7%
pH in surface horizon	6.6 – 7.3	5.0 – 6.5	5.6 – 6.0

Table 2-2. Soil physical and chemical characteristics including cation exchange capacity (CEC) with and without organic matter (OM).

	Hanlon	Clarion	Zook
	sandy loam	*loam*	*clay/clay loam*
pH	6.92	4.65	5.97
Sand (g kg^{-1})	724	445	219
Silt (g kg^{-1})	204	379	382
Clay (g kg^{-1})	72	176	400
Coarse silt (g kg^{-1})	137	212	132
Fine silt (g kg^{-1})	67	167	250
Coarse clay (g kg^{-1} of clay)	795	755	591
Fine clay (g kg^{-1} of clay)	205	245	409
CEC (no carbonates or OM) (cmol kg^{-1})	7.6	13.9	35.5
CEC (with OM) (cmol kg^{-1})	12.3	20.1	58.9
Total carbon (g kg^{-1})	7.2	18.4	30.1
Total nitrogen (g kg^{-1})	1.0	1.8	2.7
C:N (ratio)	7.3	10.0	11.0

Table 2-3. Water-dispersible colloid (WDC) organic carbon and nitrogen contents.

	Hanlon WDC	Clarion WDC	Zook WDC	Manure WDC
Total carbon (g kg^{-1})	38.9	55.9	38.1	346.0
Total nitrogen (g kg^{-1})	5.3	6.4	4.3	63.2
C:N	7.4	8.7	9.0	5.5

Table 2-4. Chemical structure and properties of 17 β-estradiol and estrone. pKa is the pH at which 50% ionization occurs. Log K_{ow} is the log of the octanol-water partitioning coefficient.

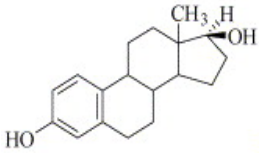
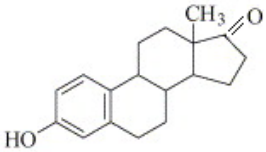
	17β-Estradiol	Estrone
Acronym	E2	E1
Structure		
Molecular mass (g mol^{-1})	272.4	270.4
Water solubility (mg L^{-1})	13-21.6	2.1-13
pK_a	10.2	10.4
Log K_{ow}	3.1-4.0	3.1-3.4

Table 2-5. Sorption parameters, K_d (partition coefficient) and log K_{oc} (organic carbon normalized partition coefficient) and correlation coefficients. Solid-to-solution ratios for each sorbent were 1:20 for Hanlon, 1:40 for Clarion, 1:60 for Zook, and 1:100 for all water-dispersible colloids (WDC).

	17 β-Estradiol			Estrone		
	K_d (L kg^{-1})	R^2	log K_{oc}	K_d (L kg^{-1})	R^2	log K_{oc}
Hanlon	15	0.98	3.32	15	0.99	3.32
Hanlon WDC	72	0.97	3.27	90	0.99	3.36
Clarion	16	0.99	2.94	18	0.96	2.99
Clarion WDC	74	0.99	3.12	108	0.99	3.29
Zook	34	0.98	3.05	31	0.99	3.01
Zook WDC	82	0.98	3.33	97	0.97	3.41
Manure WDC	213	0.99	2.79	224	0.97	2.81

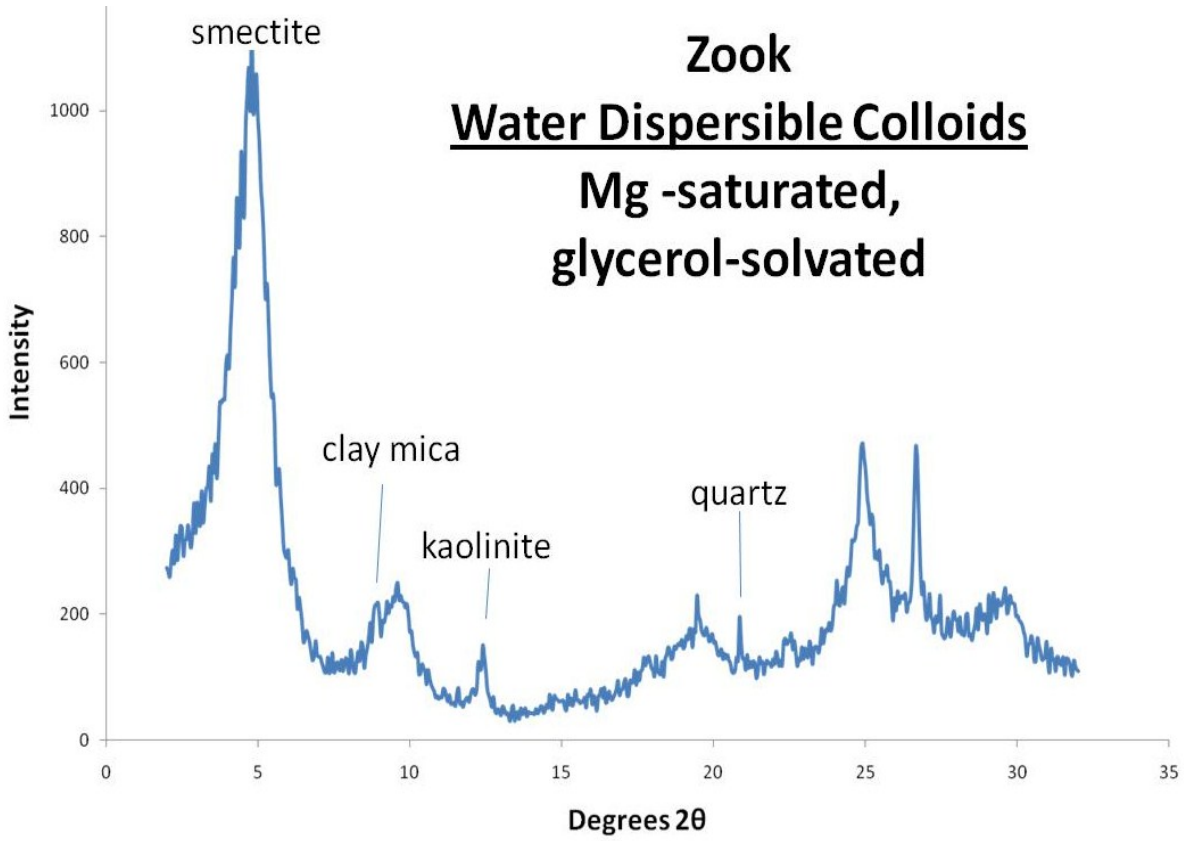


Figure 2-1. X-ray diffraction pattern of Zook water dispersible colloids delineating the major mineral components.

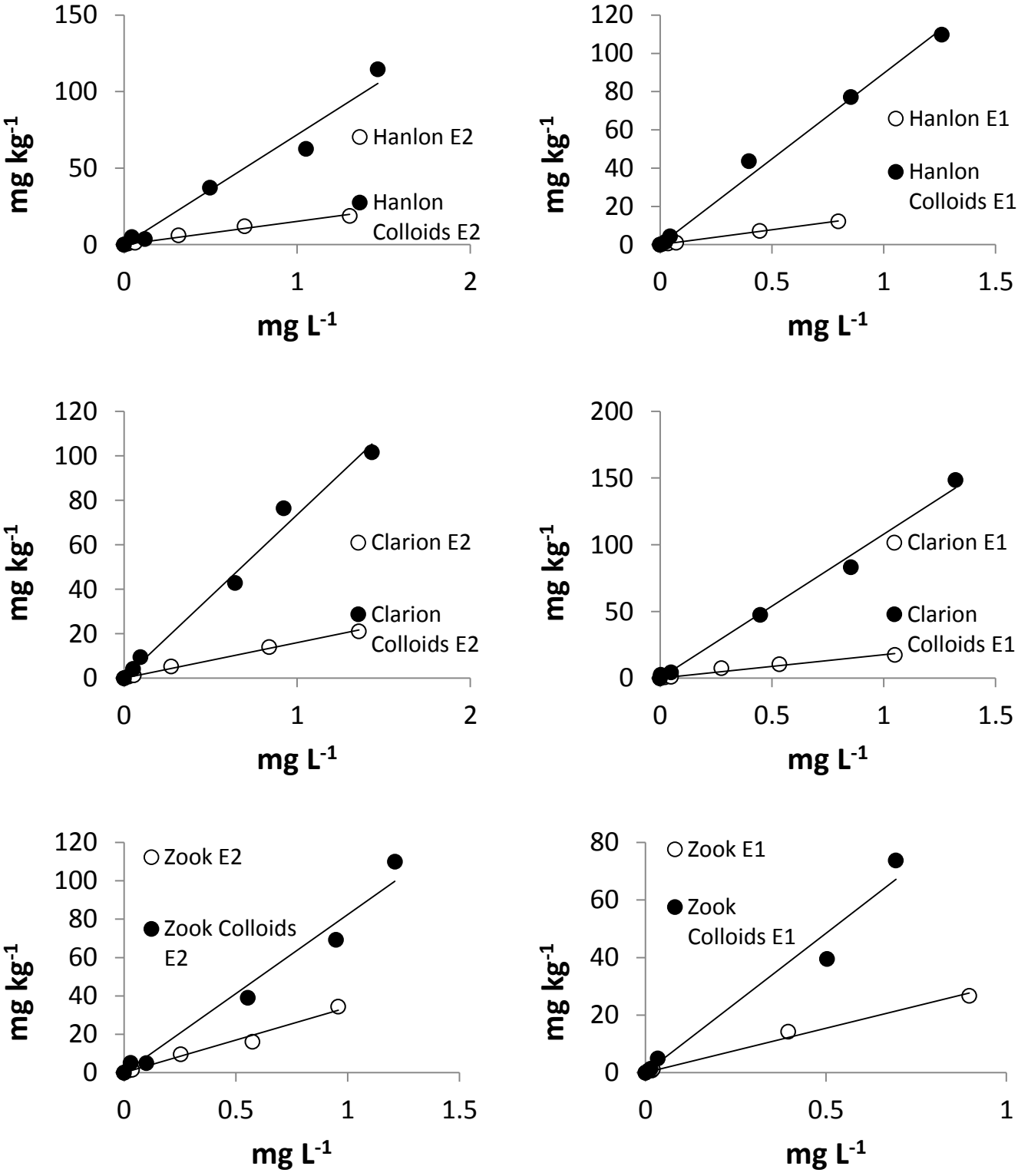


Figure 2-2. Soil and water-dispersible colloid (WDC) sorption isotherms.

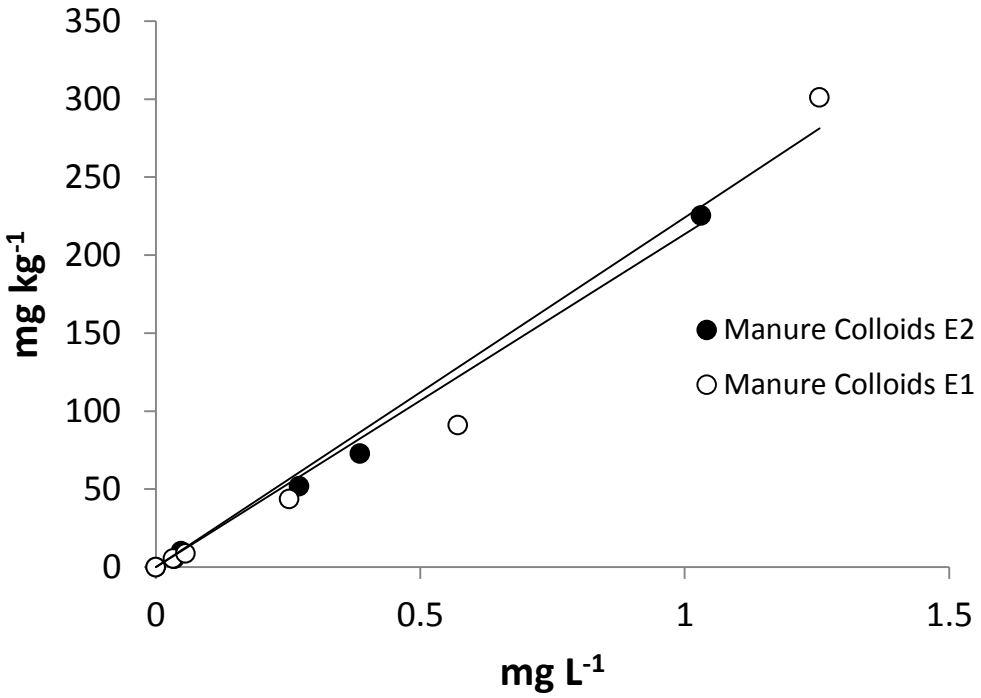


Figure 2-3. Manure colloid sorption isotherms.

Chapter 3 : Reduction of estrone to 17β-estradiol in the presence of swine manure colloids

A paper to be submitted to Journal of Environmental Quality

Jacob R. Prater, Michael L. Thompson, and Robert Horton

ABSTRACT

Land application of animal manure and municipal biosolids to improve soil fertility carries the risk of adding endocrine system disrupting contaminants to aquatic ecosystems. This study explored the fate of two estrogens, 17 β-estradiol (E2) and estrone (E1), in the presence of organic colloids derived from swine manure. Most reports concerning environmental estrogens indicate a degradation/transformation pathway that leads to decreased estrogenicity, that is E2 is transformed to E1 and then to other daughter products. However, in this study we found that within 24 hours the reverse reaction is possible (E1 transforming to E2) in a swine manure colloidal suspension closed to the atmosphere. The reaction occurred in swine manure colloidal suspensions incubated with spikes of E2 and separately with spikes of E1 after approximately the same 24-hour period. In the experiment with E2, there was a complete reversal of the solution estrogen form, from E2 to E1 and back to E2. Our observations suggest that environmental estrogens, in low oxygen conditions, may have the potential to increase in estrogenicity and/or persistence under certain conditions such as in low oxygen environments and under reducing conditions.

INTRODUCTION

Estrogens of manure origin pose a risk to surface water ecosystems due to possible endocrine-system disruption of aquatic organisms with unknown implications on higher order animals (Purdom et al., 1994; Hansen et al., 1998). Given the environmental risk posed by estrogens, it is reasonable that there is concern when they are detected where they are not expected, such as in tile drainage water, wells, and surface water. Most studies have shown strong and rapid sorption of estrogens to soil materials as well as rapid degradation of E2 to E1 and E1 to estriol (Casey et al., 2003; Lee et al., 2003; Das et al., 2004). The degradation of E2 by oxidation is of large environmental importance because E1 is about 2 to 3 times less estrogenically potent as E2 (Thorpe et al., 2003). Due to rapid sorption and degradation in a repacked column transport study, neither E2 nor E1 were readily transported through the soil column (Casey et al., 2003). In light of this, research has pointed to chemical and physical non-equilibrium conditions and/or co-transport of estrogens to explain their unexpected environmental presence. Colloidal materials have been indicated as a possible vector for co-transport of estrogens along with other possibilities (Steiner et al., 2010; Arnon et al., 2008; Casey et al., 2008).

In addition to colloid enhanced transport, soil conditions and manure application may be larger factors than previously considered when attempting to predict the fate of E2 and E1. An example of this is a field study by Kjaer et al. (2007) showing that E1 moved farther than expected and persisted longer than expected. They suggested that soil conditions, like temperature and the presence of manure could create conditions to slow the process of

degradation, lower temperatures leading to decreased reaction rates and manure application leading to lower oxygen conditions to slow degradation.

The transformation reaction of E1 to E2 has been documented in sediment and biosolids samples in very small amounts (Czajka and Londry, 2006). Also, in a laboratory study to optimize the production of E2, a yeast and E1 were used to cause this same transformation (Cheng and Tsai 2010). The persistence of estrogens under anoxic conditions and the ability to transform E1 to E2 in a laboratory setting leads to the question of whether or not a similar transformation might occur in the environment. The objective of this paper was to determine whether this reduction reaction of E1 to E2 would occur in an aqueous suspension of swine manure colloids.

MATERIALS AND METHODS

Swine manure was collected from the flushing of a farrowing house at an Iowa State University research farm. Manure colloids were operationally defined and fractionated as particles less than 1 μm equivalent spherical diameter using Stokes' Law sedimentation (assuming a particle density of $\rho_s = 1.4$ g cm^{-3}) as the upper boundary and not passing a 0.45-μm nylon filter for the lower boundary. The fractionated colloidal material from swine manure was freeze dried. Carbon and nitrogen contents of the swine manure colloidal material are presented in Table 3-1. E2 and E1 were obtained from Sigma-Aldrich (99% pure). Chemical structure and properties of E2 and E1 are presented in Table 3-2.

Batch kinetic studies were carried out to measure the rate of sorption and degradation/transformation of E2 and E1 with swine manure colloidal material. The

experiments were conducted in 50-mL glass centrifuge tubes with Teflon-lined caps at 20°C. Estrogen solutions of ~ 2 mg L^{-1} E2 or E1 were prepared in a matrix of 5 mM $CaCl_2$ with less than 1% methanol (used to aid in dissolving E2 and E1) (Prater et al., Chapter 2 this thesis). A solid-to-solution ratio of approximately 1:200 was used on the basis of estimated sorption intensities from measured organic carbon content, such that 20 to 80% of the spiked estrogen would remain in solution after sorption for ease of measurement. Incubation times ranged from 20 min to 66 h. Following incubation, each sample was centrifuged at 800 *g* for 16 min at 20 °C and an aliquot of the supernatant was adjusted to pH ~ 4 for analysis. To test the influence of oxygen on E2 and E1 transformation, a manure colloid sample was incubated in a container open to the atmosphere for 66 h prior to centrifugation.

Concentrations of E2 and E1 in solution were determined by high pressure liquid chromatography (HPLC) (Beckman System Gold model 118, 168, 507) using a pre-concentration and clean up solid-phase extraction (SPE) step (C-18 LiChrolut EN) (Xie et al., 2008), leaving the sample in methanol and acetone. A gentle N_2 gas stream at 40°C was used to evaporate the sample, and then it was resuspended in methanol. HPLC instrument parameters were: sample loop, 50 µL, flow rate, 1.5 mL min^{-1}; mobile phase, 45:55 Acetonitrile:H_20 (pH ~4); column, C-18 reverse phase (Alltima); UV detector, λ = 202 nm. HPLC parameters were similar to Lee et al. (2003). The method detection limit was 2.5 µg L^{-1} in a 30 mL sample (defined as 3 times the background signal) and the limit of quantitation was 6 µg L^{-1} in a 30 mL sample (defined as the lowest standard used). HPLC analysis was deemed more efficient for our goals of measuring degradation properties compared to slower costlier methods with lower detection limits.

RESULTS AND DISCUSSION

Upon investigating the sorption potential, kinetics, and degradation of estrogens in the presence of colloids of various origins, we discovered that the transformation of E2 to E1 is reversible in the presence of swine manure colloids. Raman et al. (2001) also suggested that this might be a possibility.

Figure 3-1 shows the time series incubation, in a closed container, of swine manure colloids with an initial spike of E2. In this incubation E2 was converted to E1, with ongoing sorption, and then after approximately 24 hours re-transformed back to E2 (Fig. 3-1). This transformation is also a complete switch over time from dominantly E2 in solution (0.3 hours), to dominantly E1 in solution (16-24 hours), and back to E2 dominating the solution phase (66 hours). There were no detectable losses of estrogen nor was there any indication that estrogen was degraded or transformed to a compound other than E2 and E1 as the percentage of added total estrogen in solution remained consistent at ~70% of the added concentration from 0.33 h until the experiment was terminated.

Similar to the experiment with an E2 spike, in a closed container E1 spiked incubation with swine manure colloids E1 began transforming to E2 after approximately 24 hours (Fig. 3-1). Both of these interactions differ from what has been reported in the field studies and in some laboratory studies. Most degradation studies of E2 and E1 report E2 transforming to E1 and then to estriol, decreasing in estrogenicity with each transformation step. This study differs in that the transformation was reversed over time. We hypothesize that oxygen was depleted in the presence of manure colloids leading to a redox potential low

enough for E1 to be reduced to E2. Due to the scent of sulfur this redox potential may have been as low as -180 to -200 mV.

To test whether anaerobic conditions were a contributing factor to this phenomenon, a manure colloid sample was incubated with E2 open to the atmosphere for 66 h (Fig. 3-1). The incubation open to the atmosphere did not produce the same result (Fig. 3-1). Instead E2 was below detection limits at 66 h and E1 was present. It appears that reducing conditions created by the microbial community and the closed atmosphere led to the reduction of E1 to E2. It is still not clear from this research whether a particular organism or just a reducing condition is necessary for this reaction.

CONCLUSIONS

Indications that E1 can be reduced to E2 in the presence of environmental colloids and reducing conditions similar to swine lagoon leachate or aquic soils presents new information for predicting the fate and transport of environmental estrogens. While the particular mechanism of this reaction E1 to E2 is not yet clear from our observations it is possible that even if both a reducing condition and a particular organism are required that the conditions could occur in the soil environment, around or beneath a swine manure lagoon, or in soil amended with swine manure. Thus, soil redox potential may be very important when attempting to predict the fate and transport of these two estrogens and potentially other similar chemicals.

The transformation and subsequent lack of degradation of the more environmentally persistent E1 to the more estrogenic E2 has important implications. There is a possibility for an increase in estrogenicity under certain environmental conditions. Moreover, there is

potential for long-range transport in anoxic conditions due to greater persistence of estrogens in anaerobic conditions. There is also potential that previous risk assessments at sites with reducing conditions have underestimated potential dissipation and transport by assuming that the degradation of environmental estrogens always proceeds in the direction of oxidation. This information suggests an alternative explanation, beyond the role of preferential flow, for why estradiol sometimes appears in groundwater (Kolodziej et al., 2004), which is that even though E2 transformation to E1 may occur in the surface soil the reverse could occur in deeper soil and in aquifers where there are reducing conditions. The management implications of this study indicate that care needs to be taken that swine manure lagoons do not contact groundwater and that newly spread manure goes onto soils that are unlikely to be water saturated and oxygen deprived soon after application.

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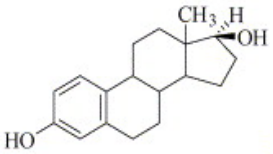
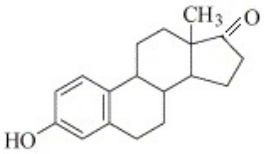
Table 3-1. Swine manure water-dispersible colloids (WDC) characteristics.

	Manure WDC
Total carbon g kg^{-1}	346.0
Total nitrogen g kg^{-1}	63.2
C:N	55

Table 3-2. Chemical structure and properties of 17 β-estradiol and estrone. pKa is the pH at which 50% ionization occurs. Log K_{ow} is the log of the octanol-water partitioning coefficient.

	17β-Estradiol	Estrone
Acronym	E2	E1
Structure		
Molecular mass (g mol^{-1})	272.4	270.4
Water solubility (mg L^{-1})	13-21.6	2.1-13
pK_a	10.2	10.4
Log KOW	3.1-4.0	3.1-3.4

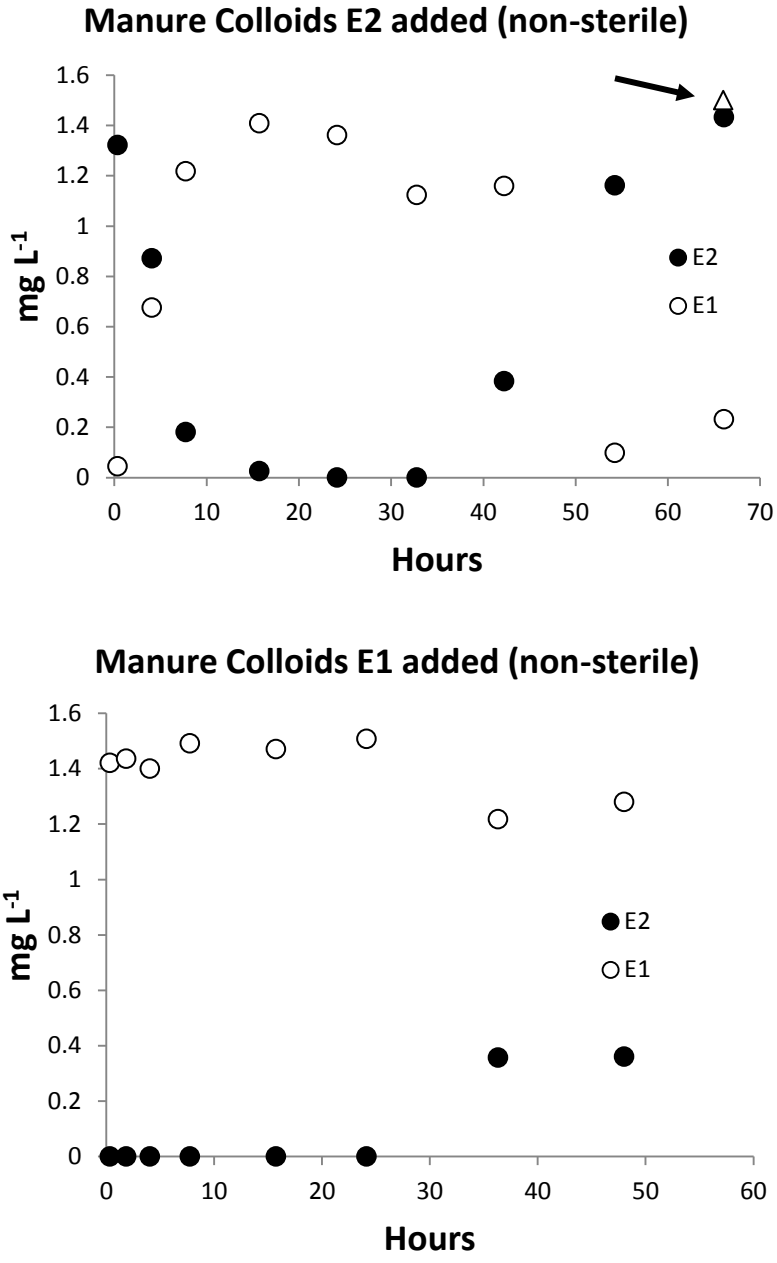


Figure 3-1. Swine manure colloid incubations (unsterilized).
The open triangle marked with an arrow in "Manure Colloids E2 added (non-sterile)" represents the E1 concentration from an incubation starting with E2 with the sample open to the atmosphere.

Chapter 4 : Sorption and degradation kinetics of 17 β-estradiol and estrone with soil materials and environmental colloids

A paper to be submitted to Journal of Environmental Quality

Jacob R. Prater, Michael L. Thompson, and Robert Horton

ABSTRACT

Endocrine-disrupting chemicals can have environmental impacts, but the mechanisms and factors controlling their dissipation in soils have not been fully investigated. The sorption and degradation kinetics of 17 β-estradiol (E2) and estrone (E1) represent important dissipation pathways of these two endocrine-disrupting chemicals. Aspects of these pathways have been investigated with soils and sediments, but there have been few studies of sorption and degradation in the context of soil colloidal material, which has been implicated in cases of unexpected transport of these two compounds in soils. In this study, sorption and degradation kinetics were measured using laboratory batch incubations of soil materials and water-dispersible colloids from soils and swine manure. The materials were spiked with ~80 μg of either E2 or E1 resulting in an initial solution concentration of ~ 2 mg L^{-1}. Samples were autoclaved (sorption only) or left untreated (sorption and degradation) and subsequently incubated with an estrogen spike in 50-mL glass tubes with Teflon-lined caps for periods ranging from 0.3 to 66 h. Solution concentrations of each compound were measured using high-pressure liquid chromatography. For both E2 and E1 the results indicate two sorption mechanisms or phases that contrasted in rate. Both sorption phases can be approximated as pseudo first-order processes, although the initial rapid phase may be more accurately

described as instantaneous. Additionally, degradation of E2 can be approximated as a first-order process with rate coefficients varying from 0.006 to 0.06 h^{-1} and with a mean of 0.026 h^{-1} (sd = 0.016) in the tested materials. The calculated half-life of E2 had a mean of 47.0 h (sd = 31.4 h) with soils and 39.8 h (sd = 37.2 h) with soil colloids.

INTRODUCTION

Estrogens such as E2 and E1 are important environmentally due to their potential to disrupt the endocrine systems of aquatic organisms such as fish and amphibians (Oberdorster and Cheek, 2000; Corcoran et al., 2010). At small (ng L^{-1}) concentrations this disruption could lead to skewed sex ratios of fish and amphibians by causing males to exhibit phenotypically female characteristics. The unknown impact of such changes on aquatic ecosystems highlights the importance of understanding the dissipation of estrogens in the environment. In general, E2 and E1 dissipate in three ways: dilution and transport, sorption, and transformation and degradation. Previous research has identified mechanisms of E2 and E1 sorption to soil which may be reversible or irreversible (Casey et al., 2003). Several studies measuring the dissipation of E2 with different soil materials suggest that dissipation can be predicted using pseudo first-order kinetics (Lee et al., 2003; Colluci et al., 2001; Ying et al., 2003).

Reported kinetic sorption rate coefficients with soils vary, but they have been approximated as pseudo first-order processes (Casey et al., 2005). Hildebrand et al. (2006) noted that sorption of E2 and E1 to soil is initially rapid and is then characterized by a slower phase of sorption. In contrast Casey et al. (2005) reported one sorption rate coefficient and one first-order process. Sorption rate coefficients for soils reported by Casey et al. (2005)

were 0.178 h^{-1} and 0.210 h^{-1} for E2 and E1, respectively. Whereas there has been evaluation of estrogen-soil interactions, these interactions have not been investigated with natural soil and manure colloidal material.

The sorption of E2 and E1 to soil has been interpreted to be dominated by hydrophobic partitioning owing to the correlation of partitioning coefficients to soil organic carbon (SOC) content (Lee et al. 2003). Still, two-site models seem to be supported by empirical data, but do not necessarily indicate different mechanisms (Casey et al. 2003). Casey et al. (2005) suggested that sorption of E2 and E1 was diffusion limited because the rate of adsorption was proportional to the square root of time. The molecular-scale mechanisms of these two apparent modes of sorption remain under debate. Perhaps two-site or two-phase behavior could be indicative of external and internal surfaces in the sorbent. This two-phase behavior could lead one to the conclusion that there is rapid external surface sorption and diffusion limited internal surface sorption, consistent with the presence of clay minerals such as smectite which have internal surfaces in their interlayer spaces. Van Emmerik et al. (2003) noted a rapid initial sorption phase and reported that E2 could intercalate into the interlayer spaces of smectite. Intercalation of E2 into expandable clay minerals suggests that even small particles like colloids can have internal spaces where diffusion could play a role in limiting the rate of adsorption or desorption of estrogens.

Reported first-order transformation and degradation rate coefficients cover a broad range. For example, Das et al. (2004) reported values of 0.0003 to 0.075 h^{-1} from a column experiment and Collucci et al. (2001) reported values of 0.013 to 0.13 h^{-1} from a batch experiment. Such ranges likely result from several factors including: experimental design (column or batch), degradation reaction model used, and soil conditions, including sample

preparation and experimental parameters. Das et al. (2004) noted that even among different column experiments there was variability in calculated degradation rates. There is evidence that these rates and the direction of the reaction may be affected by other conditions, namely the presence of oxygen and anaerobic or reducing conditions (Prater et al., 2012b; Czajka and Londry 2006; Ying et al., 2003; Cheng and Tsai 2010). Also temperature and acidity may be factors in E2 and E1 degradation, with increasing rates at higher temperatures and decreasing rates with increasing acidity (Raman et al., 2001).

There has been some evaluation of estrogen-soil interactions, but these interactions have not been investigated with soil and manure colloidal material. Colloidal materials represent a possible transport pathway for E2 and E1 in soils and a gap in our current understanding of the environmental fate of estrogens due to their high sorption capacity (Prater et al., 2012a) and their potential mobility while solution forms of E2 and E1 are removed by sorption and degradation. The objectives of this study are to compare and contrast sorption and degradation of E2 and E1 in association with soil, soil colloids, and swine manure colloids. An example schematic of the sorption and degradation process can be found in Fig. 4-1.

MATERIALS AND METHODS

Sample Collection

Three Iowa soils representing a range of particle size distributions and organic matter contents (Hanlon, Clarion, and Zook soils materials) were sampled in the top 10 cm of soil at each of three sites near Ames, IA (Tables 4-1 and 4-2). Hanlon soil material was sampled from the Iowa State Hinds Research Farm, Clarion soil material was sampled from the Iowa State Curtiss Research Farm, and the Zook soil material was sampled from the Ames, IA

Municipal Water Pollution Control Facility. The Hanlon soil is well drained, having formed in loamy alluvium, under native prairie vegetation on natural levees along streams. The Clarion soil is a well-drained upland soil formed in till under native prairie vegetation. The Zook soil is a poorly drained soil formed in silty and clayey alluvium usually in flood plains. These three soil samples provide a particle size distribution from fine sandy loam to clay loam.

Soil colloidal material was dispersed from the samples with gentle agitation in distilled water using a rotary shaker at 15 rpm for 12 h with a 1:8 soil-to-solution ratio (Kjaergaard et al., 1995). The resulting suspension was then fractionated using Stokes' Law sedimentation for particles < 1-μm equivalent spherical diameter (assuming a particle density $\rho_s = 2.65$ g cm^{-3}). The colloidal suspensions were then concentrated using auto irrigation pots (an automatic irrigation system adapted to be a porous ceramic filter apparatus) and subsequently freeze dried in preparation for further analysis.

Swine manure was collected from the flushing of a building housing sows and new piglets at an Iowa State University research farm. The colloidal material was fractionated by sieving to 53 μm; then the filtrate was further fractionated by using Stokes' Law sedimentation for particles < 1 μm equivalent spherical diameter (assuming a $\rho_s = 1.4$ g cm^{-3}). Manure colloids in suspension were concentrated on 0.45-μm nylon filters and freeze dried in preparation for further analysis.

Soil materials collected from the three sites were previously analyzed for composition and chemical attributes including particle size distribution, soil organic carbon content, pH, cation exchange capacity (CEC) measured without and without the removal of organic matter, and mineralogy (Prater et al., 2012a). Soil physical and chemical properties are listed

in Table 4-2. Water dispersible colloids (WDC) were also previously analyzed for total organic carbon and nitrogen (Table 4-3) (Prater et al., 2012a). The mineralogy of the samples was determined using x-ray diffraction (Whittig and Allardice, 1986). An example of the x-ray patterns can be found in Fig. 4-2.

Estrone and E2 were obtained from Sigma Aldrich (98% pure). Chemical structure and properties of E2 and E1 are presented in Table 4-4. 17 β-estradiol and estrone stock solutions were prepared using methanol as a co-solvent (less than 1% of the total volume), then diluted in 0.005 M $CaCl_2$. The effect of methanol as a co-solvent in limited quantities on sorption has been shown to be negligible (Bouchard 2002).

Batch Studies

Non-equilibrium, time-dependent batch studies were conducted to measure the kinetics of sorption and degradation of E2 and E1 in the presence of each of the seven soil and colloidal materials (Hanlon, Clarion, Zook, Hanlon WDC, Clarion WDC, Zook WDC, and swine manure WDC). Batch study samples were incubated in 50-mL glass centrifuge tubes with Teflon-lined caps using dried and ground soil and colloidal materials. Based on SOC content and measured sorption intensities (Prater et al., 2012a), solid-to-solution ratios were chosen (1:20 for Hanlon, 1:40 for Clarion, 1:60 for Zook, and 1:100 for WDCs) to ensure that between 20% and 80% of the added estrogen (~2 mg L^{-1}) would remain in solution to ensure measurable quantities and accuracy of measurement. Subsamples of the materials were autoclaved (60 min at 134^{o}C) to inhibit microbial activity and denature enzymes prior to incubation for sorption measurements. The samples were incubated at 20^{o}C on a reciprocating shaker with either an E2 or E1 spike. Incubation times for sorption measurements were 0.33, 2, 4, 8, 12, 24, and 48 h. Samples that were incubated to measure

degradation were not autoclaved and were incubated under the same conditions described above, but for longer duration (0.33, 2, 4, 8, 12, 24, 32, 40, 48, 56, and 66 h). Following incubation each sample was centrifuged at 800 *g* for 16 min to separate solids and solution. Then an aliquot of the supernatant was taken and adjusted to pH ~ 4 for analysis of the dissolved estrogens. All samples were replicated twice with the exception of the swine manure colloids due to their very short supply.

Analytical Techniques

Concentrations of E2 and E1 in water were determined using high-pressure liquid chromatography (HPLC). This was accomplished using a pre-concentration and clean-up solid-phase extraction with a C-18 substrate (LiChrolut EN) as per Xie et al. (2008). Samples were then evaporated under a gentle N_2 gas stream at 40^{o}C and then reconstituted in methanol. High-pressure liquid chromatography instrument parameters were: sample loop, 50 µL, flow rate, 1.5 mL min^{-1}; mobile phase, 45:55 Acetonitrile:H_20 (pH ~4); column, C-18 reverse phase (Alltima); UV detector, λ = 202 nm. Analysis with HPLC was based on the procedures used by Lee et al. (2003). The method detection limit was 2.5 µg L^{-1} in a 30 mL sample (defined as 3 times the background signal) and the limit of quantitation was 6 µg L^{-1} in a 30 mL sample (defined as the lowest standard used). HPLC analysis was deemed more efficient for our goals of measuring sorption and degradation properties compared to slower costlier methods with lower detection limits.

Calculations of Rate Coefficients and Half-Life

Sorption rate coefficients were determined empirically using linear regression of natural log transformed plots of the sorption (sterilized conditions) data. Degradation rates

were calculated using two different methods. The degradation rate of E2 to E1 was first calculated using the autoclaved sample data as a control and plotting the difference between the control and the untreated sample. An integrated form of the first-order degradation equation (Equation 1) was then used to calculate a rate coefficient using linear regression. This method of calculation assumes there is negligible desorption of E2 from the solid phase and that degradation occurs primarily in the dissolved phase.

$$(E_{2i} - (E_{2sp} - E_{2d})) = E_{2i}e^{-k_{deg}t} \quad [1]$$

In Eq. 1, E_{2i} (mg L^{-1}) was the initial solution phase E2 concentration, E_{2sp} (mg L^{-1}) refers to the data from sterile sorption incubations, E_{2d} (mg L^{-1}) refers to the concentration from untreated degradation incubations, k_{deg} (h^{-1}) is the degradation rate coefficient, and *t* is time. The second method used to calculate E2 degradation to E1 was from the E1 data obtained from the degradation experiments. In this calculation we assume rapid sorption of E1 and a nearly instant equilibrium of E1 in solution with E1 in the sorbed phase. The calculation uses Eqs. 2 and 3 and include the use of a partitioning coefficient (K_d value) determined in earlier work (Prater et al., 2012a).

$$E_{2i}e^{-k_{deg}t} = (E_{2i} - \left(E_{1s}\frac{M}{V} + E_{1d}\right)) \quad [2]$$

$$K_d = \frac{E_{1s}}{E_{1d}} \quad [3]$$

In Eq. 2, E_{2i} (mg L^{-1}) was the initial solution phase E2 concentration, E_{1s} (mg kg^{-1}) is E1 on the solid phase, M (kg) is the mass of soil, V (L) is the volume of solution, E_{1d} (mg L^{-1}) is E1 in the solution phase of the degradation experiment, and E_{2i} (mg L^{-1}) is the E2 added initially. Equation 3 is a calculation of the E_{1s} for the measured partition coefficient (K_d) from Prater et al., 2012a. Half-lives were then calculated from each of the above rate coefficients using

Equation 4. These calculated half-lives represent a range of the half-life of E2 having been calculated with slightly different assumptions that may overestimate or underestimate half-life.

$$t_{\frac{1}{2}} = \frac{\ln 2}{k_{deg}} \qquad [4]$$

In Eq. 4 $t_{1/2}$ is the half-life.

RESULTS AND DISCUSSION

Autoclaved samples exhibited no signs of degradation metabolites, so it was assumed that these treated samples had no degradation occurring. Both E2 and E1 were rapidly sorbed by soil and colloidal materials in autoclaved samples, approaching equilibrium after 10 to 12 h (Fig. 4-3). Equilibrium in the sorption of both E2 and E1 was reached by 24 hours, as reported by Xie et al. (2008) and Lee et al. (2003). Amin et al. (2012) noted that apparent sorption equilibrium was reached more rapidly with swine manure materials than with soil materials. This more rapid sorption to swine manure materials appears to be the case in the present experiments as well (with the exception of E2 with soil materials) with E2 and E1 reaching equilibrium with swine manure colloids within 8 h (Fig. 4-3). The longer equilibration time of E2 and E1 with soil and soil colloidal materials (12 h) could be due to the presence of minerals, namely smectite found to be the dominant clay mineral in these materials (Prater et al., 2012a), creating lower accessibility sorption sites. These lower accessibility sites might include hydrophobic nanopores in clay quasicrystals, sub-stack surfaces, similar to what was described by Hundal et al. (2001) when investigating the

sorption of phenanthrene to reference smectites. Van Emmerik et al. (2003) has suggested that E2 sorption to montmorillonite occurs in interlayer spaces and required a period of about three days of contact to reach equilibrium indicating that some sorption sites may require considerable time for reactions to occur.

Soil materials as well as soil and swine manure colloids exhibit two-phase sorption for both E2 and E1 (Fig. 4-3). Due to the lack of data points prior to 0.33 h, it is difficult to characterize the initial rapid sorption phase because its endpoint is not clearly defined from this data. Because of this uncertainty, only the rate coefficients of the second phase are reported. In order to make comparisons among the sorbents, boundaries were set to determine a reaction rate for the slower sorption phase. The boundaries used were the measurement at 0.33 h, where there is a clear change in rate and at 95% of the total sorption during the incubation, a proxy for equilibrium. Pseudo first-order rate constants (k_2 h^{-1}) for the second phase of sorption differed among the sorbents, with a mean of 0.066 h^{-1} (sd = 0.04) for E2 with soil materials, 0.060 h^{-1} (sd = 0.02) for E2 with soil colloidal materials, 0.035 h^{-1} (sd = 0.015) for E1 with soil materials, and 0.047 h^{-1} (sd = 0.006) for E1 with soil colloidal materials (Table 4-5). If it were calculated from the two measurements available (0 and 0.33 h), the initial, rapid phase had a rate coefficient as much as one order of magnitude larger than the second phase. It is possible that this initially rapid phase may be hard to distinguish under different experimental conditions (timing of sampling, initial concentration, and solid-to-solution ratio) which could lead to lumping both sorption phases.

The two sorption phases may differ mechanistically/chemically, with one being more rapid than the other. But such an interpretation may be overstating what occurs. Amin et al. (2012) suggested the possibility that the two phases of sorption represent similar chemical

reactions that occur at exterior surfaces and interior surfaces. In other words, there may be one mechanism, hydrophobic partitioning, but access varies to different sorbing surfaces, exterior and interior, with diffusion limiting the rate of the interior surface sorption. Van Emmerik et al. (2003) noted that montmorillonite could sorb E2 and that it could be intercalated into interlayer spaces of clay. The potential for intercalation of E2 into expanding clay minerals may provide some insight concerning the two apparent phases or mechanisms of sorption. Organomineral colloids (both fractionated and present in the unfractionated soil materials) consist of organic matter with clay and could provide interior spaces for a chemical to diffuse into and sorb. Additionally, these interior spaces may consist of hydrophobic nanopores in clay quasicrystals, sub-stack surfaces, similar to what was described by Hundal et al. (2001). A third possibility is that there are microaggregates present that contain interior surfaces with organic coatings. The presence of interior sorption sites might also explain some of the apparent irreversible sorption or incomplete recovery reported by Lee et al. (2003).

The proportions of sorbed E2 and E1 for each of the two phases were calculated as the mass sorbed during each phase relative to the total amount sorbed. The proportions of sorption in these phases show some variability across materials. The first phase constituted 32 to 83% of the total amount sorbed and the second phase constitutes 17 to 68% of the total amount sorbed (Table 4-6). The initial rapid phase represented a large proportion of the total sorption of both E2 and E1 to the soil materials and swine manure colloids while the soil colloids appeared to have more sorption in the slower phase than the other materials (Table 4-6). The larger proportion of sorption in the slower phase for soil colloids could be due to the greater clay content of these materials, creating more interior sorption surfaces where

diffusion limited the rate of sorption. A larger array of soils with varying clay content could be used to test this hypothesis.

The presence of two distinct sorption phases differs from the work of Casey et al. (2003) who reported just one pseudo first-order process. In the present study, initial concentrations were at least one order of magnitude higher and solid-to-solution ratios were smaller than in the work of Casey et al. (2003). Additionally, the sorption rate coefficients reported by Casey et al. (2005) (0.178 h^{-1} and 0.210 h^{-1} for E2 and E1, respectively), using a soil similar to Hanlon in organic matter content, were also greater than the slow phase measured in this work.

The E2 degradation data reveal similar sorption behaviors to those measured separately in autoclaved samples, initially rapid then slowing before degradation dominates instead of equilibrium being reached (Fig. 4-3, 4-4, and 4-5). Unsterilized samples show a first-order degradation of E2 to E1 after sorption approached the equilibration time of the sterile samples (Fig. 4-4, and 4-5). Concomitant sorption and degradation resulted in a rapid initial decrease in solution E2 in conjunction with an increase in solution E1 followed by a slower decrease in E2 concentration approaching zero alongside a plateauing E1 solution concentration. This is similar to what Raman et al. (2001) reported from incubations of E2 with dairy manure. These results, showing degradation of E2 to E1 in a 3-day period, differ from those reported by Casey et al. (2005), which showed no degradation of E2 (i.e., no metabolites present in solution) in a similar period. Instead of E2 liquid concentrations approaching zero as in this study, Casey et al. (2005) measured E2 concentrations that approached an equilibrium.

Similar to the results of Raman et al. (2001) there was a small re-emergence of E2 in some samples toward the end of the incubation (data not shown), but the concentrations remained below the quantification limits used in this study (6 μg L^{-1} in a 30-mL sample). The re-emergence of E2 could be due to sorption hysteresis (slow release of E2 from the solid phase after solution-phase degradation) or to a reaction similar to that described by Prater et al. (2012b), i.e., reduction of E1 to E2 under reducing conditions. If the re-emergence of E2 is due to a desorption process, then we may infer that degradation took place preferentially in the solution phase rather than the sorbed phase, which is contrary to the assumption of Casey et al., 2005. This conclusion follows from the rapid rate of sorption and the lack of competition between E2 and E1 for sorption sites on soils containing organic matter noted by Bonin and Simpson (2007). If degradation occurred on the solid phase, desorption of E2 would be unlikely, because removal of E2 from the solid phase would presumably lead to disequilibrium resulting in more sorption of E2 rather than desorption. This interpretation does not mean that degradation was not associated with the solid phase, but rather that degradation did not occur primarily in the sorbed phase. In order to better understand the complex kinetics of E2 and E1 removal from the system, either the sorbed phase must also be measured to account for this desorption or a reliable model must be constructed to describe the behavior.

When the degradation rate of E2 was calculated using the two methods described previously (Equations 1-3) a range of degradation rate coefficients was produced varying from 0.006 to 0.06 h^{-1} with a mean of 0.026 h^{-1} (sd = 0.016) (Table 4-5). These rate coefficients, k_{deg}, were similar to those reported by Das et al. (2004), 0.0003 to 0.075 h^{-1} from a column experiment and Collucci et al. (2001), 0.013 to 0.13 h^{-1} from a batch

experiment. Half-lives of E2 calculated with Equation 4 range from 11.6 to 115.5 h with a mean of 43.4 h (sd = 34.6) (Table 4-5). Reported degradation rates are variable, revealing that soil conditions are an important factor.

CONCLUSIONS

Both E2 and E1 were rapidly removed from the solution phase by sorption processes in these soil and manure derived materials. Degradation further removed E2 from solution in the soil and colloidal materials. These experiments did not indicate any degradation of E1, as E1 concentrations in degradation experiments plateaued or appeared to be approaching an equilibrium. The measured kinetics of E2 and E1 sorption showed a rapid phase and a second slower phase in all materials measured. We hypothesize that these phases arise from exterior and interior surfaces and that the two rates derive from surface sorption and diffusion-limited sorption. This hypothesis arises from the presence of smectite in the mineral fractions of all the studied materials, with exception of the swine manure, and the work of Van Emmerik et al. (2003) showing intercalation of E2 into smectite. Testing of this hypothesis might require characterization of interior and exterior surface areas as well as the effective diffusion coefficients of these materials (a difficult task) and/or the use of manufactured materials with known interior and exterior surface areas.

Degradation of estrogens may be rapid in soil under aerobic conditions, but it ceases and can even reverse under anaerobic conditions (Prater et al., 2012b). 17 β-estradiol has a mean half-life of 47.0 h (sd = 31.4 h) with soils and 39.8 h (sd = 37.2 h) with soil colloids, as calculated from measurements in this study. Due to rapid sorption and a short half-life, E2 is not likely to be transported in soils unless conditions allow for reduced soil contact times or

decreased degradation. If degradation primarily occurs in the solution phase, then sorption may protect E2 from some forms of degradation. This makes colloids especially important in the potential transport of E2. Colloids have a high capacity for sorption of estrogens and thus have potential to protect estrogens from degradation during transport. Additionally, the initial rapid sorption phase indicates an area of further study due to its greater importance under physical and chemical non-equilibrium transport conditions. Under these conditions, soil contact times may be short and sorption rates developed from longer incubation times may underestimate sorption.

Conditions favorable to E2 and E1 transport include: anaerobic conditions resulting in decreased degradation rates, preferential flow leading to decreased soil contact times, and the presence of suspended organic colloids protecting E2 and E1 from degradation during transport. Therefore, applying E2-containing manure to soils when wet conditions might ensue could risk transport of estrogens. Thus in order to decrease the risk of estrogen transport, soil contact time and oxygen availability should be maximized when applying manure which may contain high levels of estrogens. In doing so sorption and degradation will be promoted and transport and leaching reduced. Thus, applications of manure known to have high estrogen content should be applied in drier conditions when soil gas exchange is high and soil water movement is slow, especially avoiding areas where there is shallow groundwater, tile drainage systems, or karsts.

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Table 4-1. Soil characteristics.

Soil	Hanlon	Clarion	Zook
Classification	Coarse-loamy, mixed, superactive, mesic Cumulic Hapludoll	Fine-loamy, mixed, superactive, mesic, Typic Hapludoll	Fine, smectitic, mesic Cumulic Vertic Endoaquoll
Parent material	Alluvium	Till	Colluvium/Alluvium
Surface horizon texture	Fine sandy loam	Loam	Silty clay loam
Landscape position	Floodplains	Convex uplands	Floodplains
Slope	0 – 2%	2 – 5%	0 – 2%
Drainage class	Moderately well drained	Well drained	Poorly drained
Organic matter in surface horizon	1 – 3%	3 – 4%	5 – 7%
pH in surface horizon	6.6 – 7.3	5.0 – 6.5	5.6 – 6.0

Table 4-2. Soil physical and chemical characteristics including cation exchange capacity (CEC) without and without organic matter (OM) removal.

	Hanlon	Clarion	Zook
	sandy loam	*loam*	*clay/clay loam*
pH	6.92	4.65	5.97
Sand (g kg^{-1})	724	445	219
Silt g (kg^{-1})	204	379	382
Clay (g kg^{-1})	72	176	400
Coarse silt (g kg^{-1})	137	212	132
Fine silt (g kg^{-1})	67	167	250
Coarse clay (g kg^{-1} of clay)	795	755	591
Fine clay (g kg^{-1} of clay)	205	245	409
CEC (no carb or OM) (cmol kg^{-1})	7.6	13.9	35.5
CEC (with OM) (cmol kg^{-1})	12.3	20.1	58.9
Total carbon (g kg^{-1})	7.2	18.4	30.1
Total nitrogen (g kg^{-1})	1.0	1.8	2.7
C:N (ratio)	7.3	10.0	11.0

Table 4-3. Water-dispersible colloid (WDC) chemical properties.

	Hanlon	Clarion	Zook	Swine Manure
Total carbon (g kg^{-1})	38.9	55.9	38.1	346.0
Total nitrogen (g kg^{-1})	5.3	6.4	4.3	63.2
C:N	7.4	8.7	9.0	5.5

Table 4-4. Chemical structure and properties of 17 β-estradiol and estrone. pKa is the pH at which 50% ionization occurs. Log K_{ow} is the log of the octanol-water partitioning coefficient.

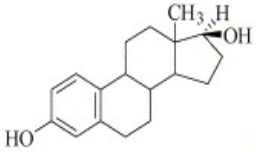
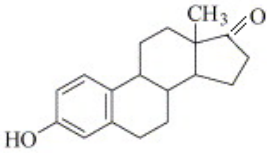
	17β-Estradiol	Estrone
Abbreviation	**E2**	**E1**
Structure		
Molecular mass (g mol^{-1})	272.4	270.4
Water solubility (mg L^{-1})	13-21.6	2.1-13
pK_a	10.2	10.4
Log K_{ow}	3.1-4.0	3.1-3.4

Table 4-5. Rate coefficients (k_2) for the second phase of sorption, degradation rate coefficients (k_{deg}) calculated two ways (from E2 solution measurements and from E1 solution measurements), and half-lives ($t_{1/2}$) for soils, soil water-dispersible colloids (WDC) and swine manure (SM) WDCs. Correlation coefficients (R^2) are in parentheses.

	17 β-Estradiol					Estrone
	k_2 (h^{-1})	k_{deg} (h^{-1}) Eq 1 (R^2)	k_{deg} (h^{-1}) Eq 2 (R^2)	$t_{1/2}$ (h) Eq 1	$t_{1/2}$ (h) Eq 2	k_2 (h^{-1})
Clarion	0.050	0.018 (0.91)	0.019 (0.88)	39	37	0.031
Hanlon	0.029	0.023 (0.96)	0.034 (0.98)	30	20	0.019
Zook	0.120	0.006 (0.96)	0.017 (0.71)	116	41	0.055
Clarion WDC	0.026	0.012 (0.91)	0.041 (0.93)	58	17	0.055
Hanlon WDC	0.070	0.032 (0.63)	0.045 (0.92)	22	15	0.047
Zook WDC	0.083	0.006 (0.87)	0.060 (0.92)	116	12	0.040
SM WDC	0.067	-	-	-	-	0.053

Table 4-6. Proportions of E2 and E1 in each phase (1, initial rapid phase and 2, second slower phase) of sorption as a proportion of the total amount sorbed.

	E2		E1	
Sorption Phase	1	2	1	2
Clarion	0.74	0.26	0.67	0.33
Hanlon	0.83	0.17	0.62	0.38
Zook	0.66	0.34	0.77	0.23
Clarion WDC	0.43	0.57	0.59	0.41
Hanlon WDC	0.34	0.66	0.57	0.43
Zook WDC	0.32	0.68	0.68	0.32
Manure WDC	0.65	0.35	0.75	0.25

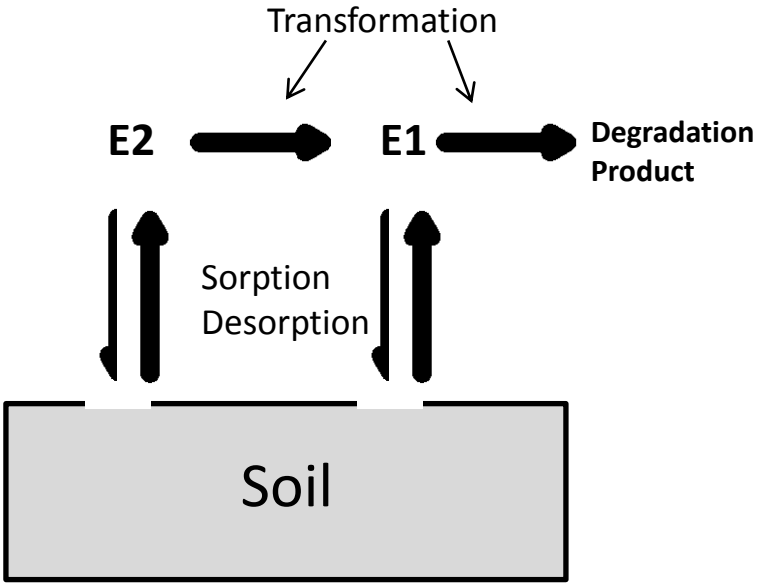


Figure 4-1. Conceptual model of E2 and E1 reaction processes with soil.

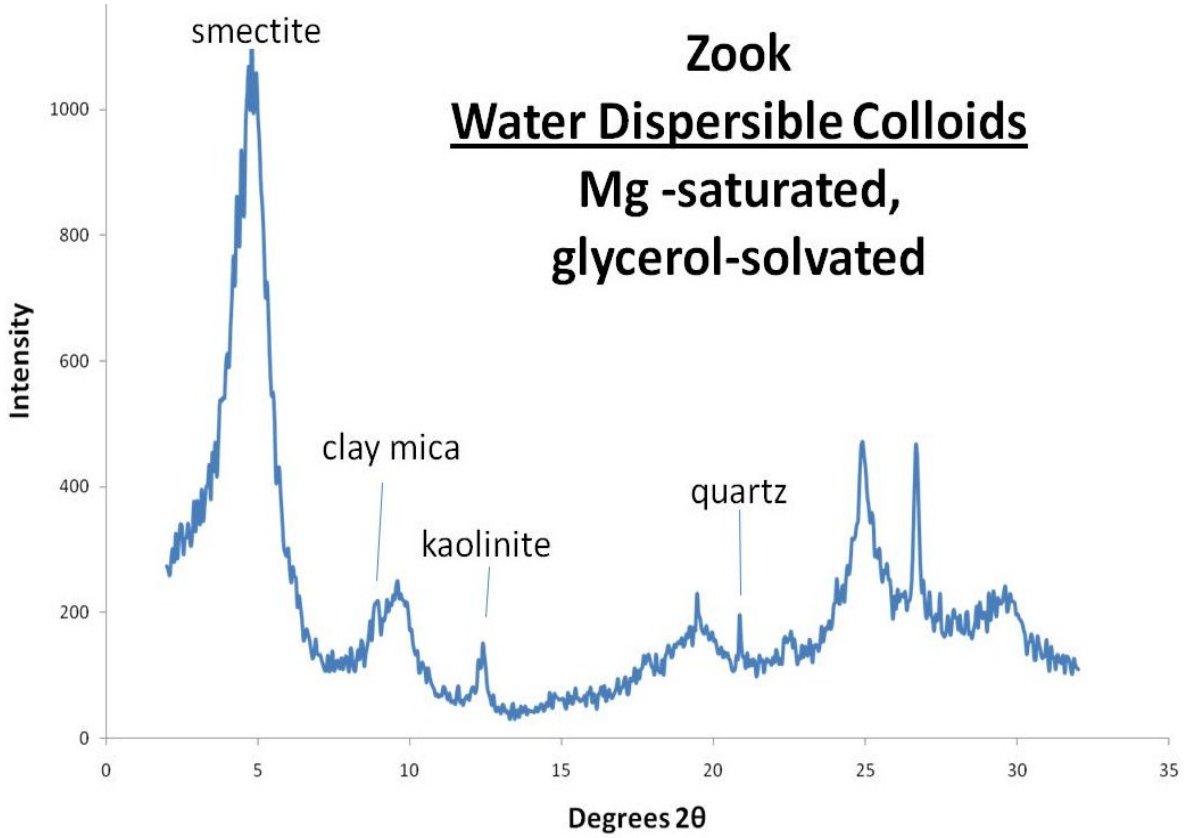


Figure 4-2. X-ray diffraction pattern of Zook water dispersible colloids delineating the major mineral components.

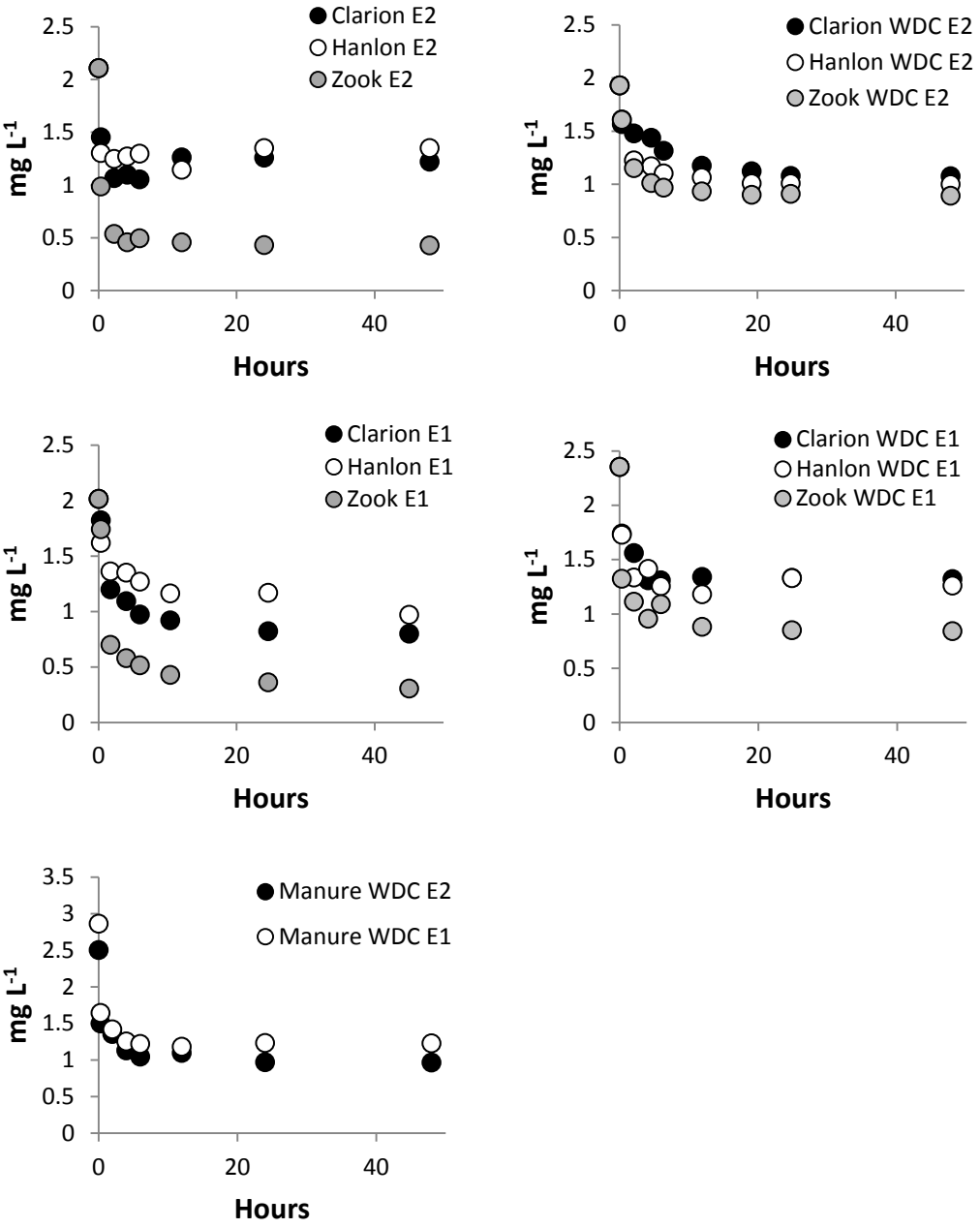


Figure 4-3. Sorption kinetics of 17 β-estradiol (E2) and estrone (E1) with soil, soil water-dispersible colloids (WDCs), and swine manure WDCs.

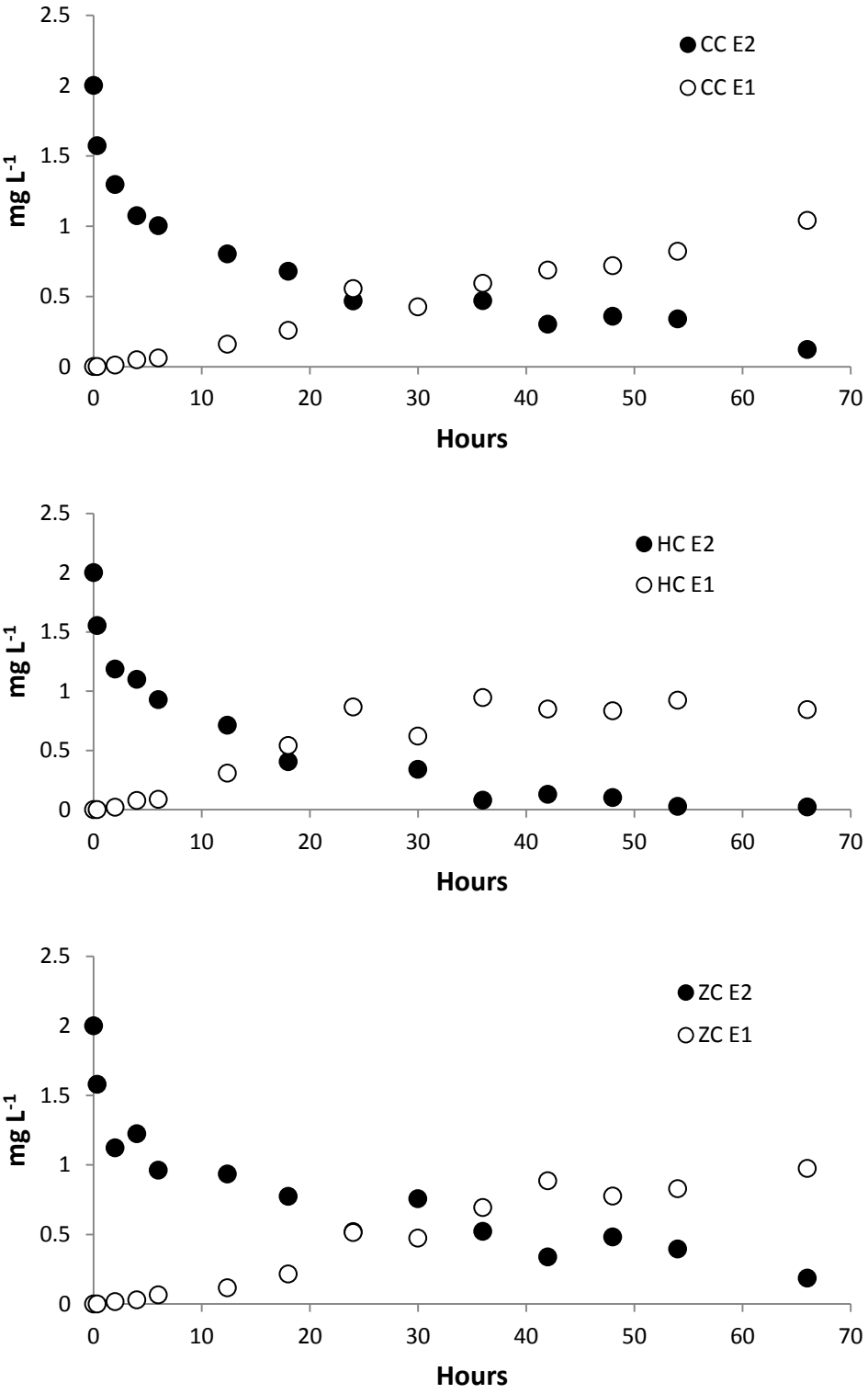


Figure 4-4. Degradation of 17 β-estradiol (E2) to estrone (E1) with soil water-dispersible colloids (WDC). CC, HC, and ZC are Clarion WDCs, Hanlon WDCs, and Zook WDCs respectively.

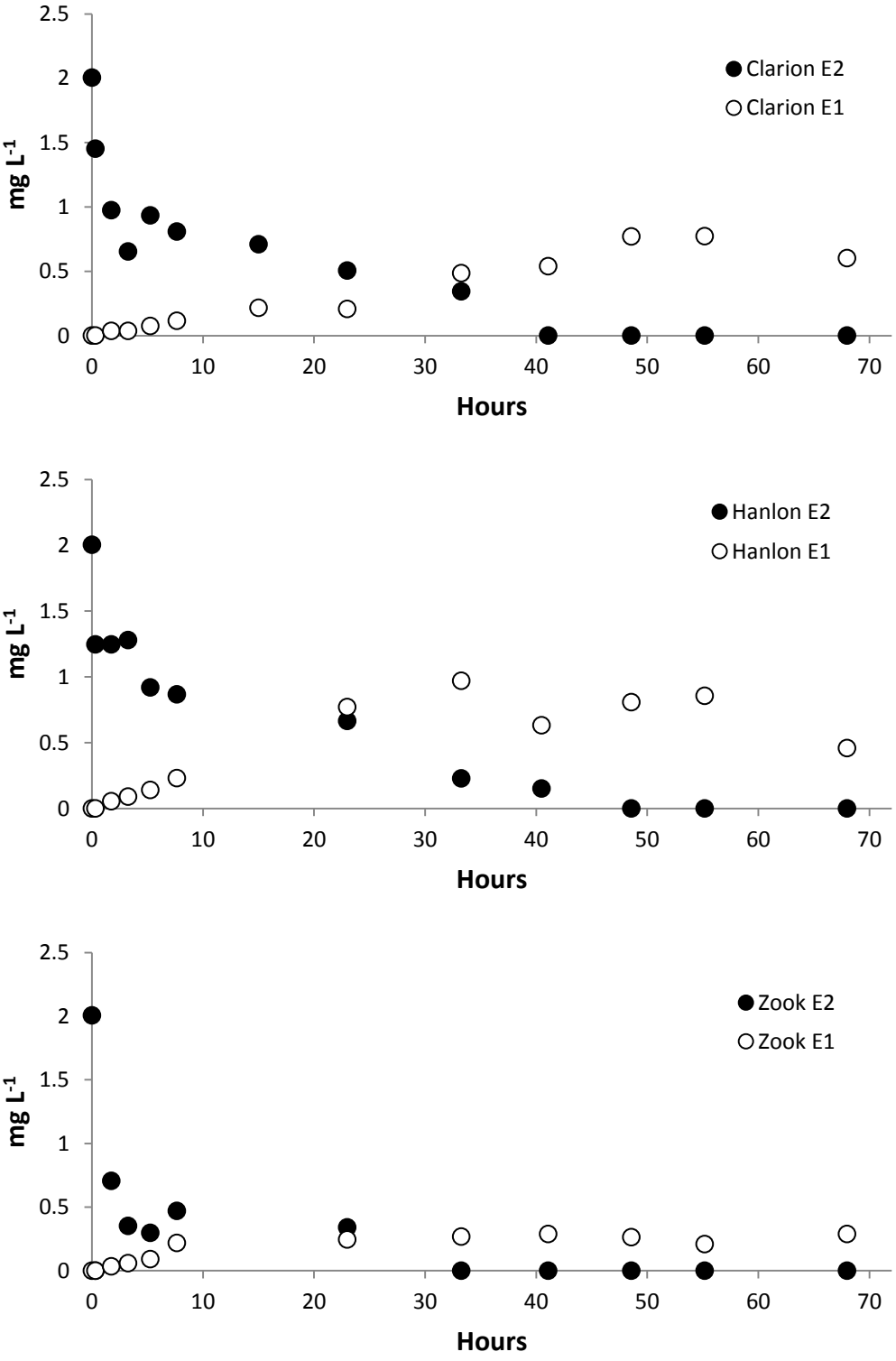


Figure 4-5. Degradation of 17 β-estradiol (E2) to estrone (E1) with soil.

Chapter 5 : Impacts of environmental colloids on the transport of 17 β-estradiol in intact soil cores

A paper to be submitted to Journal of Environmental Quality

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ABSTRACT

Estrogens such as 17-β estradiol (E2) are endocrine disrupting compounds and may have biological effects on aquatic organisms. Therefore, it is important to understand the mechanisms of their transport in the environment. E2 and its daughter product estrone (E1) are both strongly sorbed by soil organic matter and have relatively short half-lives (11-120 hours), provided oxygen is available. Thus, conditions that reduce contact time with soil make transport in soil more likely. In this study, intact soil cores from three Iowa soils were used to compare the transport of E2 with and without the presence of colloidal material fractionated from soil or swine manure. In chemical transport experiments conducted with soil columns, E2 and E1 were measured both in solution and attached to suspended solids in column effluent by using high-pressure liquid chromatography (HPLC). Colloids were transported through all but one column and carried sorbed E2 with them in all cases of transport. The presence of colloids decreased the first detection time of E2 relative to a bromide tracer in all three soils. The presence of colloids was also correlated with greater peak E2 concentrations in eluent in all but one soil column. Additionally, the presence of colloids resulted in a greater mass fraction of E2 to be transported in solution. The presence

of suspended colloids with sorbed E2 led to more rapid E2 transport in solution, larger amounts of E2 transport in solution, and transport of E2 sorbed to mobile colloids.

INTRODUCTION

There is growing awareness of the negative effects of estrogens and other endocrine disruptors on aquatic organisms (Oberdorster et al. 2000, Corcoran et al. 2010). This has led to concern about the environmental impacts of the use of estrogenic hormones, for livestock production and human contraceptives, and other endocrine-disrupting chemicals (EDCs) and the materials that naturally contain them (animal manures and biosolids). EDCs can have biological effects at very low concentrations. For example, E2 levels of 1 ng L^{-1} or less have been discovered to cause males of a certain fish species to produce vitellogenin, a protein associated with egg production (Purdom et al., 1994 and Hansen et al., 1998). Additionally, these chemicals have been shown to occur in waste water effluent and biosolids, apparently escaping degradation or removal during treatment (Aga, 2008). E2 is also present in livestock manure from concentrated animal feeding operations (CAFOs); this manure is normally not given secondary treatment before land application (Aga, 2008; Lange et al., 2002). Thus, understanding the fate of estrogens is important for improving management of biosolids and manure to maintain water quality and healthy aquatic ecosystems. A current area of inquiry is the co-transport of estrogens with colloids.

Colloids are often defined as being < 2 μm in diameter and larger than material that is truly dissolved. Colloid-facilitated transport of organic contaminants is a phenomenon that has been reported for a number of years. McCarthy and Zachara (1989) explored many of

the possible factors in colloid facilitated transport of contaminants in the subsurface and indicated many areas where research might be pursued. Sojitra et al. (1995) noted that the transport of hydrophobic organic contaminants can be linked to mobilized colloids. Some examples of co-transport of organic contaminants with colloids include: Vinten et al. (1983) who reported that DDT was transported through soil on suspended soil and organic particles, Seta and Karathanasis (1997) who reported that atrazine could be transported by suspended soil colloids, and Villholth et al. (2000) who reported that prochloraz could be transported by suspended particles. In most cases colloid-facilitated transport is considered possible if the compound of interest has a high K_d (partitioning coefficient) value, thus predicting limited aqueous-phase transport. Conditions that might make colloid-facilitated transport more likely include the possibility that the sorbed phase is protected from degradation, sorption hysteresis (with slower desorption), and the presence of well-connected macropores in the soil. Prater et al. (2012c) suggested that sorption may protect E2 from degradation, or at least that degradation occurred primarily in the liquid phase, and that desorption of E2 from soil was inferred to be slower than adsorption. Research in waste water treatment has indicated that E2, sorbed to colloidal organic carbon, can be carried through the treatment process (Holbrook et al., 2003).

E2 and E1 exhibit a strong affinity to sorb to soil organic carbon (SOC); log K_{oc} (organic carbon normalized K_d) values are reported to be in the range of 2.3 to 4.2 L kg^{-1} for E2 and from 2.5 to 4.1 L kg^{-1} for E1 (Lee et al., 2003; Ying et al., 2003; Casey et al., 2005; Sangsupan et al., 2006; Sarma et al., 2008; Xie et al., 2008; Prater et al., 2012a). Log K_{oc} values for E2 and E1 with suspended river colloids (3.95 L kg^{-1} for E2 and 4.18 L kg^{-1} for E1 (Liu et al., 2005)) are similar to log K_{oc} values measured with wastewater colloids (4.08 to

4.68 L kg^{-1} for E2 (Holbrook et al., 2003)) both of which are greater than those measured in soils. The sorption of estrogens to soil and colloidal materials has been described as rapid (Lee et al., 2003; Casey et al., 2005; Prater et al., 2012c), leading to the conclusion that transport of estrogens is only likely when macropores contribute to flow (leading to physical and chemical non-equilibrium conditions). The reason for this conclusion is two-fold. First, in the absence of colloids only rapidly moving soil solution with minimal soil contact would be capable of carrying the estrogens an appreciable distance before they were sorbed and/or degraded. Second, it is likely that colloids can facilitate the transport of estrogens only in relatively large pores where the colloids themselves are less likely to be retained or filtered out by the soil material. These considerations make it unlikely that estrogens or similar compounds would be transported in significant quantities in unsaturated soils. Under varying conditions (column length, diameter, and flow rate), transport experiments with E2 in soil have produced different results. Relatively small soil columns used by Casey et al. (2005) (7-cm length) and Das et al. (2004) (10-cm length) resulted in significant transport of E2, while larger columns like those used by Casey et al. (2003) (15.2-cm length) and Fan et al. (2008) (30-cm length) resulted in little to no E2 transport.

Colloid transport has been reported to be more rapid than the transport of a conservative tracer such as Br^-. It is suggested by Poulsen et al. (2006) that this enhancement in transport velocity is a result of size exclusion, that is, the colloids are excluded from some of the smallest soil pores due to their size and thus do not travel in the slowest flowing small pores present in the soil. The presence of colloidal material offers a means for estrogen transport that allows for more rapid movement (via size exclusion) and protection from degradation (due to sorption). Thus, the potential for colloid facilitated transport of E2 poses

a unique problem for predicting arrival times and mass movement of chemicals like estrogen due to the as yet unpredictable interaction of E2 with colloids during transport.

Casey et al. (2005) reported that there was discrepancy between E2 sorption and degradation parameters measured with soils in static systems and those calculated from observations of E2 transport in repacked soil columns. Further there is a large difference in arrival times of E2 in repacked soil columns and intact soil columns (Casey et al., 2005; Fan et al., 2008). The difference in E2 arrival times between repacked columns and intact soil columns has led to the inference that estrogen transport in soil could be enhanced by preferential flow or possibly colloid mediation. It is likely that both preferential flow (from soil aggregation and macropores) and the presence of suspended colloidal material may affect E2 transport in soil leading to a possible enhancement of E2 transport. Kjaer et al. (2007) noted a pronounced macropore flow effect on E2 and E1 transport to tile drains suggesting that manure application to structured soils presented a risk to surface waters receiving tile drainage effluent. At least one field study has indicated that colloids may facilitate the transport of E2 through soil (Casey et al., 2008).

Previous studies of estrogen-soil interaction have included batch sorption (Lee et al. 2003), disturbed and undisturbed soil column experiments (Das et al., 2004; Fan et al., 2008), and field scale studies (Kjaer et al., 2007; Casey et al., 2008). None of these, however, have attempted to determine the effect of the mobile colloidal phase on the mobility and transport of E2 and E1. This mobile solid phase may have a considerable impact on assessing the environmental risks of E2 application with soil amendments of manures or biosolids.

The aqueous solubilities of E2 and E1 are affected by pH, ionic strength, and temperature. At typical environmental pHs, E2 and E1 are expected to be non-ionized since

their pK_as are greater than 10 (Table 5-1). High ionic strength has the effect of coagulating organic compounds by 'salting out,' thus decreasing their solubility (Shareef et al., 2006). Due to the potential effects of ionic strength on the solubility of E2 and E1 and stability of the colloidal suspension, a solution of low ionic strength that mimics the soil solution phase is suited for transport experiments.

The present study was designed to investigate how suspended colloidal material might enhance estrogen transport. Colloidal material, operationally defined as soil and manure particles smaller than 1-μm equivalent spherical diameter (ESD), has the potential to enhance estrogen transport in two ways: by providing a mechanism for rapid transport and by protecting the estrogens from biodegradation during transport (Prater et al., 2012c). Thus colloids may facilitate estrogen transport to locations where desorption could occur leading to adverse biological and ecological effects. Hildebrand et al. (2006) demonstrated that desorption of E2 from the soil can occur in water if enough contact time is allowed. There are other effects to consider beyond rapid sorption, degradation, and preferential flow in the investigation of E2 and E1 fate and transport. One must also take into account factors that may enhance or influence transport and those that suspend or inhibit degradation, such as anaerobic conditions and low temperatures (Prater et al., 2012b; Raman et al., 2001). In the experiments performed for this paper temperature was controlled and oxygen was at ambient conditions.

The role of organic colloids in inhibiting estrogen degradation has been noted by wastewater treatment engineers who discovered that the amount of colloidal organic material in wastewater effluent affected its estrogenic activity (Holbrook et al., 2003). Because estrogens can be both sorbed and degraded in soil (Lee et al., 2003, Das et al., 2004, Colluci

et al., 2001, and Prater et al., 2012a and c) the most likely mode of transport of estrogens to surface waters through soils may be colloid facilitated transport.

As the impacts of EDCs in the environment are recognized it is important to investigate the nature and mechanisms of their fate and transport in soils, which is best accomplished through a multi-scale study involving equilibrium sorption, kinetics, and column-transport experiments. There is presently limited continuity or replication in such studies. While several transport mechanisms have been investigated in packed and intact soil columns, with aqueous estrogen forms, and co-transport with dissolved organic carbon (urine) revealing some of the behavior of estrogens in soils, colloid mediated transport of estrogens has not been extensively investigated (Das et al., 2004; Casey et al., 2005; Fan et al., 2008; Lucas and Jones, 2009). Due to the prevalence of structured soils, the high sorption capacity of colloidal material for E2 and E1 (Prater et al., 2012a), and the rapid rates of degradation and sorption of E2 and E1 (Prater et al., 2012c) it is likely that colloid facilitated transport of E2 may be a real phenomenon worth investigating due to the potential for more rapid E2 transport and longer duration of E2 persistence during transport in soil. Because of the concern for enhanced E2 transport via colloid facilitation, the objectives of this paper are to determine if suspended colloids contribute to or enhanced the transport of E2 (affecting arrival time, peak delivery, or mass fraction transported) and its primary metabolite E1 in intact soil cores representing a range of textures and organic carbon contents.

MATERIALS AND METHODS

Chemicals

Both E1 and E2 were obtained from Sigma Aldrich (98% pure). Estrogen stock solutions were prepared using methanol as a co-solvent (less than 1%) and then diluted in 1 mM $CaBr_2$. The effect of methanol as a co-solvent in limited quantities on sorption has been shown to be negligible (Bouchard 2003).

Sample Collection

Three Iowa soils (Clarion, Hanlon, and Zook) representing a range of particle size distributions and organic matter contents were included in this study. Intact cores from three sites near Ames, IA were collected from each of these soils. Clarion (fine-loamy, mixed, superactive, mesic Typic Hapludoll) soil cores were collected from the Iowa State University Curtiss Farm, Hanlon (coarse-loamy, mixed, superactive, mesic Cumulic Hapludoll) soil cores were collected from the Iowa State University Hinds Research Farm, and the Zook (fine smectitic, mesic Cumulic Vertic Endoaquoll) soil cores were collected from the Ames Municipal Water Pollution Control Facility. The Hanlon soil is well drained, having formed in loamy alluvium under native prairie vegetation on natural levees along streams. The Clarion soil is a well-drained upland soil formed in glacial till under native prairie vegetation. The Zook soil is a poorly drained soil formed in silty and clayey alluvium usually in flood plains. These three soil samples provide a particle size distribution from fine sandy loam to clay loam along with a broad spectrum of permeability. Nine undisturbed cores (10 cm diameter, 20 cm length) were obtained from each of these soils for transport experiments.

Colloidal material was fractionated from previously collected soil and swine manure (Prater et al., 2012a). The soil colloidal material was fractionated through gentle agitation using a rotary shaker at 15 rpm for 12 hours with a 1:8 soil to solution ratio using distilled water (Kjaergaard et al., 1995). The resulting suspension was then fractionated by using Stokes' Law of sedimentation for particles less than 1-µm equivalent spherical diameter (assumed particle densities were, $\rho_s = 2.65$ g cm^{-3} for soil and $\rho_s = 1.4$ g cm^{-3} for swine manure). The soil colloidal suspensions were concentrated by using a porous ceramic filter apparatus and subsequently freeze dried. The manure colloids were concentrated on 0.45-µm nylon filters instead of the auto irrigation pots due to their much smaller quantity and to avoid fouling the equipment. Following this step, the manure colloids were also freeze dried.

Chemical and physical characterization methods used on the soils and colloidal materials are reported elsewhere (Prater et al., 2012a) and the results are summarized in Tables 5-2 and 5-3. The particle size distributions of colloidal material were measured using laser scattering (Cilas, model 990 Orleans, France) with mean particle sizes and percentage in size classes found in Table 5-4.

Column Studies

Nine intact soil cores (diameter, 10 cm; length, 20 cm) were collected from each of the three sampling locations. The saturated hydraulic conductivity of each core was measured, and from each soil, three cores with similar saturated hydraulic conductivity were selected for chemical transport experiments. Average hydraulic conductivity of Clarion cores was 0.4 cm min^{-1} and 0.9 cm min^{-1} for Zook soil cores. Hanlon soil cores had to be treated differently from others. Initial transport experiments with Hanlon soil cores yielded little or no E2 or E1 in the effluent after 15 pore volumes. For this reason, a subsampling of

the larger cores was conducted to allow measurements to be made. These subsampled cores were 4.3 cm in diameter and 10 cm in length.

The column studies were conducted with bromide (Br^-) as a conservative tracer in tandem with a pulse of E2 and with or without WDCs from soil or manure. Bromide was measured using a flow-through ion-selective electrode (Microelectrodes Inc., Bedford, New Hampshire). Operation of the Br^- electrode was well above its detection limit of 10^{-5} M (±2%). Bromide measurements were used as a basis of comparison against E2 and to compare physical non-equilibrium conditions between columns. Colloids in effluent were measured in line using a spectrophotometer with a flow-through cell measuring light attenuation at ν = 400 nm, calibrated with prepared colloidal suspensions. A fraction collector collected fractions of the effluent for estrogen analysis. Thus, Br^- and colloid concentration could be measured in the effluent without sacrificing any of the sample needed for estrogen analysis. A diagram of the experimental apparatus is in Fig. 5-1.

Columns were saturated over a period of 24 h from the bottom up using the resident electrolyte solution (0.25 mM $CaBr_2$ and 0.75 mM $CaCl_2$). Flow was then initiated in the upward direction under a hydraulic gradient of about three in order to facilitate the removal of entrapped air. When bubbling ceased at the top of the core, the flow direction was reversed to the downward flow direction, and the column was flushed with the resident solution for at least 10 pore volumes. A steady flow rate of 4 – 5 mL min^{-1} was then established using a clamp on the outlet tubing to control flow. After achieving steady flow, a fraction collector was used to collect fractions of no greater than 0.1 pore volumes each. For the smaller Hanlon cores fractions of less than 0.5 pore volumes were used. A Br^- and E2 pulse, with or without soil or manure colloids, was then applied for approximately 2 pore

volumes. The initial concentrations of E2 were 2 mg L^{-1} for the condition of no colloids and 4 mg L^{-1} when colloids were present. These concentrations were chosen to correspond to previous batch experiments of equilibrium sorption and kinetic sorption and degradation (Prater et al., 2012a and c). The 4 mg L^{-1} with WDCs was chosen as it would result in a solution concentration of ~2 mg L^{-1} after equilibration with colloids and thus be similar to the condition without colloids present.

In transport experiments with colloids, the colloids were previously autoclaved (60 min at 134^{o}C), sonicated to re-suspend, and allowed to equilibrate with the E2 solution on a stir plate (24 h at 20^{o}C). Initial colloid concentrations in the leaching suspensions were 3 g L^{-1} for soil-derived colloids and 0.3 g L^{-1} for manure-derived colloids. The tracer pulse was followed with at least 10 pore volumes of the resident solution (up to 50 pore volumes for the Hanlon cores).

Collected effluent fractions containing colloids were centrifuged (800 *g* for 16 min) as soon as possible to separate solid and solution. E2 and E1 were measured in an aliquot of the solution using a pre-concentration and clean-up solid-phase extraction (SPE) step (C-18 LiChrolut EN) resulting with the analytes in a methanol and acetone solution (Xie et al., 2008). A gentle N_2 gas stream at 40^{o}C was then used to evaporate the solvent before the analytes were dissolved in methanol.

The solid-phase portion of the centrifuged fraction was freeze dried and subsequently extracted with methanol (24 h on a shaker at 20^{o}C) with a solid-to-solution ratio of 85 mL of methanol to each gram of solid to ensure reproducible recovery. Recovery rate of E2 and E1 from colloids was 64%, ± 5% at $\alpha = 0.05$ (95% confidence interval). The volume of methanol used in each centrifuged fraction was adjusted based on colloid concentrations

measured with the spectrophotometer to meet the 1:85 solid-to-solution ratio. To concentrate the sample for analysis, an aliquot of the methanol extract was evaporated under N_2 gas at 40^{o}C to approximately 1 mL.

E2 and E1 in methanol were then determined by using high-pressure liquid chromatography (HPLC) (Beckman System Gold). High-pressure liquid chromatography instrument parameters were: sample loop, 50 µL, flow rate, 1.5 mL min^{-1}; mobile phase, 45:55 Acetonitrile:H_2O (pH ~4); column, C-18 reverse phase (Alltima); UV detector, ν = 202 nm. These parameters were similar to those used by Lee et al. (2003). The method detection limit was 2.5 µg L^{-1} in a 30 mL sample (defined as 3 times the background signal) and the limit of quantitation was 6 µg L^{-1} in a 30 mL sample (defined as the lowest standard used). HPLC analysis was deemed more efficient for our goals of measuring transport properties compared to slower, costlier methods with lower detection limits.

RESULTS AND DISCUSSION

According to laser diffraction particle size measurements the water-dispersible colloids fractionated from soil and swine manure were larger than the intended <1-µm (Table 5-4). The mean particle diameter of swine manure colloids (21 µm) was larger than colloids derived from soil (3-4 µm). The discrepancy between the sizes intended to be fractionated and those measured by laser diffraction is most likely explained by the assumptions each method makes. Stokes' law of sedimentation and laser diffraction both assume that particles are spheres, but this assumption leads to two different types of possible error. In Stokes' Law sedimentation, this means that non-spherical particles of larger size may behave as if

they were smaller spheres. Thus, some particles > 1μm were included in the fractionated material. The assumption of spherical particles in the laser scattering experiments can lead to imperfect results as well. The orientation of an individual particle can lead to its size measurement being different. For example, an oblong particle with its long axis parallel to the laser will appear smaller than when its long axis is normal to the laser. The second discrepancy in size between swine manure colloids and soil colloids may be explained by an incorrect assumption of density or shape, leading to differently sized particles being fractionated.

The impact of preferential flow cannot be overstated when dealing with suspended colloids and rapidly sorbing chemicals. A preliminary experiment in this study using a packed sand column with no continuous cracks or channels showed that very few colloids were transported; most of the colloids added to the top of the column were retained in the top few centimeters of sand (data not shown). Further evidence of the role of preferential flow and soil structure in the transport of colloids and E2 came from the Hanlon soil cores which exhibited very little structure. The original 10-cm diameter Hanlon core yielded almost no detectable E2 or E1 in the column effluent. In this coarse-textured, weakly structured soil with relatively high organic matter content, there was little transport of E2 or E1, whether colloids were present or not.

Clarion loam

With only bromide and E2 added, the Clarion soil core exhibited a significant delay in E2 and E1 breakthrough compared to the bromide (Br^-) tracer (Fig. 5-2). Effluent concentrations of the two compounds E2 and E1 peak at approximately 1.5% of the added E2 concentration, and then displayed significant tailing, with E1 exhibiting a longer tail than E2.

The presence of E1 indicated that E2 was transformed into E1 in the soil column. The delay in arrival and small peaks of both compounds indicate that sorption and degradation were occurring in the soil column. This agrees with rapid sorption and degradation reported in batch studies with this same soil material (Prater et al., 2012c). In contrast, Clarion soil cores with either soil colloids or manure colloids exhibited a much earlier arrival of E2 relative to the Br^- tracer (Table 5-5 and Figs. 5-3 and 5-4). The arrival of E2 in the cases with added colloidal material was coincident with or earlier than the Br^- tracer and at similar relative concentrations as the Br^-. The peak E2 concentration in the Clarion core with soil colloids added approached 90% of the added E2 concentration. With swine manure colloids, the peak E2 concentration approached 50% of the added E2 concentration. In conjunction with this large solution-phase peak of E2, there was also a colloid-associated (sorbed) E2 peak, showing that colloids can carry E2 with them through a soil column (Figs. 5-3 and 5-4). Colloids that were transported through the soil columns had sorbed concentrations that were not different from those of the added sorbed concentration. Some colloids, however, were retained in the soil column. E2 on these retained colloids may have (through desorption) made some contribution to the prolonged tailing of both E2 and E1 in these experiments.

Mass fractions of E2 transported in solution form were higher in the Clarion columns with added colloids than the in the column without colloids added (Table 5-6 and Figs. 5-12, 13, and 14). However, in the Clarion column with only Br^- and E2 added there was a greater mass fraction of E1 (as a fraction of the added E2) transported than E2. It is likely that this trend toward more E2 being transported in solution, both as expressed in peak concentration eluted and mass fraction eluted, is due to some desorption of E2 from the added colloidal material. The negligible difference between sorbed E2 concentrations on eluted colloids and

sorbed E2 concentrations on added colloids, suggests that any E2 being contributed into the solution phase from colloids is likely from colloids retained by the soil column.

Hanlon sandy loam

With only Br^- and E2 added to a Hanlon soil core, a modest peak of E2 was eluted with a maximum concentration of 7% of the added concentration (Fig. 5-5). This experimental condition also yielded a delayed and very broad E1 peak of similar amplitude to the E2 peak. The first detection times of E2 and E1 were significantly delayed behind the Br^- tracer by approximately three pore volumes.

In the Hanlon soil core with soil colloids added, E2 appeared in the effluent earlier than in the previous case with no colloids (Fig. 5-6). With soil colloids present, E2 was first detected at 0.8 pore volumes, the peak E2 concentration was less than two pore volumes out of phase with the peak Br^- concentration, and the peak E2 concentration approached 20% of the initial concentration (Fig. 5-6). Similar to the Hanlon core with only Br^- and E2 added, the Hanlon core with soil colloids added exhibited a very broad E1 peak, but with much more extensive tailing. Only a small amount of colloidal material was transported through this particular column and so a very small amount of E2 was transported in the sorbed phase. Compared to the no-colloids case there was some enhancement in transport of E2 in the presence of soil colloids. The time of first detection was earlier (Table 5-5) and the mass fraction transported as E2 in solution was greater (Fig. 5-15 and Table 5-6).

In the Hanlon core with swine manure colloids, there was no measurable E2 in the effluent and only a small peak for E1 that was significantly delayed behind the Br^- tracer (Fig. 5-7). It is likely that this core had weakly expressed soil structure and few if any macropores which would explain the lack of E2 transport, colloid transport, and colloid

facilitated transport of E2. It is likely that this lack of rapid flow paths would allow for more soil contact time leading to more sorption and degradation of E2 and E1. If there were no continuous macropores present, then it is likely that the swine manure colloids would be retained in the pores of the upper portion of this soil, similar to the results of the preliminary study using a sand column.

Zook clay loam

In Zook soil cores with only Br^- and E2 added there was a slight delay in E2 elution behind Br^-. In the columns with colloids added, E2 was eluted at nearly the same time as Br^- (Table 5-5). In the columns with colloids added, there was a greater peak E2 concentration and mass fraction of E2 in solution transported (Table 5-6 and Figs. 5-8, 9, and 10). The relative pore volumes for Br^- relative concentration = 0.5 were very similar (Table 5-5).

With the three Zook columns representing the most physically similar set of soil columns in this (similar Br^- first detection times, and similar times of Br^- $C_r = 0.5$) study some specific observations can be made (Tables 5-5). The Zook column with only Br^- and E2 added differed from the column with Br^-, E2, and soil WDCs added, indicating that there was some enhancement of transport by soil WDCs (Figs. 5-8 and 5-9). This enhancement was in the form of a three-fold increase in the peak E2 solution concentration. There was not a large difference in the eluted E1 concentrations among the columns. In addition to enhanced transport in the liquid phase, the colloids themselves carried E2 through the column. Moreover, the mass fraction of E2 transported in the solution phase in the column with soil colloids added was nearly four times larger than it was in the column without added colloids (Table 5-6). There was also an indication of enhanced E2 transport in the Zook column with added swine manure colloids. In that column the peak concentration of effluent

E2 was twice that of the column with only Br^- and E2 added (Figs. 5-8 and 10). Also in that column, the mass fraction of E2 transported in solution was almost three times greater than in the column without added colloids (Table 5-6). Taken together, the three observations of earlier times of first detection, the larger mass fraction of solution E2 transported, and the fact that colloids were transported with sorbed E2 suggested that suspended colloidal material had a profound effect on E2 fate and transport in the Zook soil columns.

Comparisons Across Soils

Comparisons across all soils show some consistent trends. In soil cores with only Br^- and E2 added, there was a significant delay in E2 and E1 compared to the Br^- tracer (Figs. 5-2, 5-5, and 5-8 and Table 5-5). In all soils both E2 and E1 also exhibited smaller peak concentrations when added in the absence of added colloids than when added with colloids. Additionally, in all of the soils there was a larger mass fraction of E2 transported in the solution phase when colloids were added and transported (Table 5-6). The exception to this is the Hanlon soil column with added swine manure colloids which resulted in no E2 or measurable colloids in the eluent.

In general, in the mass fraction assessment, soil colloids were responsible for more transport of E2 than swine manure colloids. This is a direct result of a greater mass fraction of the added colloidal mass being transported, as the E2 sorbed-phase concentrations on the colloids were not different from the initial E2 sorbed-phase concentrations on colloids. The difference in the E2 mass fraction transported sorbed on colloids, soil versus swine manure, is likely due to the size difference in the colloidal materials, where swine manure WDCs had a mean diameter of 21 μm while the soil WDCs had a mean diameter range of 3 – 4 μm (Table 5-4 and 6). Perhaps even more explanatory of the discrepancy in E2 sorbed-phase

mass fraction transport is the percentage of particles < 5 μm in diameter. In the soil WDCs, the percentage < 5 μm is from 70 to 86% while in the swine manure WDCs it is about 13% (Table 5-4). There may be other factors to consider in explaining this apparent discrepancy, e.g., the density of the material, mineral content (as it affects charge and the likelihood of adherence to soil), and any affinity of bacteria for soil surfaces.

The presence of colloids enhanced overall transport, but also increased soluble E2 transport. We hypothesize that colloids deposited on pore walls became a source of E2. This phenomenon of colloid transport wherein deposited colloids become a deposited source of a chemical being transported was also invoked by Albarran et al. (2011) in a study of strontium transport on bentonite colloids in a constructed granite fracture. However, in contrast to the results of Albarran et al. (2011), the presence of colloids in soils in this case decreased the time of first detection and increased the relative concentration of the applied chemical emerging from the column at its peak by a factor of 20 to 50. The phenomenon of colloids being retained and leading to the deposition of an E2 source in the soil is further corroborated by the photo in Fig. 5-11, showing deposited colloids lining a macropore in a Clarion soil column. This process of colloidal material forming linings inside macropores has been noted in pedology for many years. Once deposited, E2-containing colloids may release (desorb) E2 as the soil solution concentration is lowered via sorption, degradation, and transport processes. Hildebrand et al. (2006) noted that estrogens such as E2 and E1 could be desorbed from soil and sediment materials. Thus, deposited E2-containing colloids, can buffer the solution concentration in macropores via desorbing E2 and facilitate soluble E2 transport in the faster portion of the soil flow regime.

The enhancement of E2 transport due to the presence of colloidal material is displayed in the data in Table 5-5. In the soil columns with only Br^- and E2 added there is a distinct delay in the time of first detection of E2 versus Br^-. The delay in E2 first detection is not as large in the Zook soil cores. Across all soil columns the first detection of Br^- is very similar (with the exception of Hanlon columns due to their high flow rate to pore volume ratio Table 5-5). In contrast to the soil columns without added colloids, all of the soil columns where colloids are added and eluted, Br- and E2 are detected at nearly the same time. Further indication of the role of suspended colloid material in this earlier arrival of E2 is illuminated by the similar times of first detection of the colloidal materials to those of E2 and Br^-. Using the relative pore volume (RPV) at one half relative concentration (C_r) as a proxy for preferential flow (values < 1 indicate preferential flow) reveals that in at least one case (Clarion with added swine manure colloids) there can be an enhancement effect on E2 transport without an indication of preferential flow (Table 5-5).

Figs. 5-12 through 5-20 show the mass fractions of E2 and E1 as proportions of E2 added, E2 on colloids, and Br^- as a function of RPV. In all cases 100% (± 5%) of Br^- was transported through each column after 12 pore volumes (Table 5-6). Among soils the mass fraction of E2 transported is higher in the columns with colloids added and transported than in those without added colloids (Table 5-6). In soil columns without colloids added the mass fraction of E1 eluted was greater than E2 (Figs. 5-12, 5-15, and 5-18). It is likely that some of the increase in E2 eluted from the columns with colloids added was due to desorption of E2 from colloids transported part way through the columns and then deposited. The presence of colloids (of soil or swine manure origin) with E2 sorbed on them contributed to greater mass fractions of E2 transported in these three soils.

CONCLUSIONS

Colloid transport in soil has long been recognized as an important soil process, but only in recent decades has it been considered as a process for contaminant movement and co-transport. It is important to remember that the presence of colloids is not an environmental problem despite their capability to facilitate contaminant transport. Colloids and their movement in a soil profile can contribute to more uniform distributions of nutrients and organic matter with depth. They are also important in the form of microorganisms transported to the subsurface where they immobilize nutrients and aid in the dissipation of contaminants. The presence of colloids with sorbed E2 under saturated flow conditions did lead to greater transport of estrogens. Rather than seeking to control or reduce colloid transport, a short sighted approach, it might make better sense to consider reducing erosion and managing manure more efficiently to combat the issues presented by colloid facilitated transport of E2 or other chemicals found in manure.

Depending on the presence of preferential flow channels this can occur in the form of colloid facilitated transport and increased solute transport or as increased, but delayed miscible transport if the colloids already have sorbed estrogens and are retained at or near the soil surface.

Colloidal material can carry estrogens through soil, and preferential flow pathways do contribute to colloid transport, but do not seem to be a requirement for colloid transport. When present, colloids can facilitate the movement of soluble estrogens via a hitchhiking pathway where estrogens are carried partway on colloids and then become entrapped and

desorb estrogen. The presence of colloids during transport decreased the time of first detection as compared to a Br^- tracer and increased the concentration of E2 at peak delivery as well as the total E2 solution mass fraction of solution phase E2 delivered in column effluent.

The implications of this suggest that in situations where a contaminant such as E2 or E1 is present, and there are preferential flow paths, that measures should be taken to control the suspension of colloids and their introduction to the soil from runoff/erosion of manure or bio-solids. Preferential flow pathways can also contribute to transport of E2 or E1 in soils with significant organic matter present. However, this may not be the case for very low organic matter soils as noted previously that transport was not retarded nor was there any trace of degradation in a clean packed sand column. The real contrast in transport comes from comparing columns both containing preferential flow pathways one having colloids added and the other not. In this comparison the colloids can have a very significant impact on E2, and likely E1 transport if present in sufficient quantity. This condition is possible in a location receiving manure runoff from a feedlot or on a heavily manure amended field.

Perhaps another way to address this issue would be to reduce the usage of estrogens as livestock supplements (lowering the concentrations of estrogens in manure) and manage manure more agronomically (leading to smaller amounts added per land area) with a focus on building soil. These two changes along with conserving soil would effectively remove the problem of agriculturally related estrogens entering surface waters from existence. This would be accomplished by lowering the estrogen concentration in manure, preventing erosion losses of manure offsite, and having a more dilute application of manure to land reducing the risk of estrogens, other contaminants, or nutrients from entering surface waters.

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Table 5-1. Chemical structure and properties of 17 β-estradiol and estrone. pKa is the pH at which 50% ionization occurs. Log K_{ow} is the log of the octanol-water partitioning coefficient.

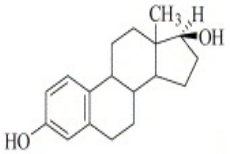
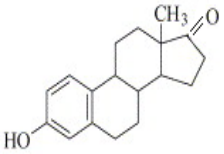
	17β-Estradiol	Estrone
Acronym	E2	E1
Structure		
Molecular mass (g mol^{-1})	272.4	270.4
Water solubility (mg L^{-1})	13-21.6	2.1-13
pK_a	10.2	10.4
Log K_{ow}	3.1-4.0	3.1-3.4

Table 5-2. Soil physical and chemical characteristics including cation exchange capacity (CEC) with and without organic matter (OM).

	Hanlon	Clarion	Zook
	sandy loam	*loam*	*clay/clay loam*
pH	6.92	4.65	5.97
Sand (g kg^{-1})	724	445	219
Silt (g kg^{-1})	204	379	382
Clay (g kg^{-1})	72	176	400
Coarse Silt (g kg^{-1})	137	212	132
Fine Silt (g kg^{-1})	67	167	250
Coarse Clay (g kg^{-1} of clay)	795	755	591
Fine Clay (g kg^{-1} of clay)	205	245	409
CEC (no carbonates or OM) (cmol kg^{-1})	7.6	13.9	35.5
CEC (with OM) (cmol kg^{-1})	12.3	20.1	58.9
Total carbon (g kg^{-1})	7.2	18.4	30.1
Total nitrogen (g kg^{-1})	1.0	1.8	2.7
C:N (ratio)	7.3	10.0	11.0

Table 5-3. Water-dispersible colloid (WDC) organic carbon and nitrogen contents.

	Hanlon WDC	Clarion WDC	Zook WDC	Manure WDC
Total carbon (g kg^{-1})	38.9	55.9	38.1	346.0
Total nitrogen (g kg^{-1})	5.3	6.4	4.3	63.2
C:N	7.4	8.7	9.0	5.5

Table 5-4. Soil and swine manure water-dispersible colloid (WDC) size distributions from laser scattering measurements.

	Hanlon WDC	Clarion WDC	Zook WDC	Manure WDC
Mean diameter	4 μm	4 μm	3 μm	21 μm
% under 2 (μm)	32	39	51	4
% under 5 (μm)	70	70	86	14
% under 25 (μm)	100	100	100	70
% under 50 (μm)	100	100	100	91

Table 5-5. Chemical transport experiment relative pore volumes (RPV) of arrival, peak, and relative concentration equal to 50% (C_r = 0.5). Data for Bromide (Br^-), 17 β-estradiol (E2), swine manure (SM) water-dispersible colloids (WDC), and soil WDC.

	Conditions	First Detection E2	First Detection Br^-	Br^- C_r = 0.5	First Detection WDC	Peak E2 WDC
		----------RPV----------				
Clarion	Br^- & E2	2.23	0.06	0.74	-	-
	Br^-, E2, & soil WDC	0.07	0.09	0.45	0.01	1.59
	Br^-, E2, & SM WDC	0.06	0.04	1.05	0.04	1.76
Hanlon	Br^- & E2	2.8	0.28*	0.96	-	-
	Br^-, E2, & soil WDC	0.79	0.25*	0.85	0.37	4.7
	Br^-, E2, & SM WDC	-	0.31*	0.69	-	-
Zook	Br^- & E2	0.15	0.06	0.43	-	-
	Br^-, E2, & soil WDC	0.07	0.09	0.49	0.07	2.05
	Br^-, E2, & SM WDC	0.06	0.08	0.52	0.06	1.42

Values with an asterisk (*) are labeled and differ from others due to the higher ratio of flux to pore volume as a result of the smaller core size used for Hanlon soil columns.

Table 5-6. Mass fraction of 17 β-estradiol (E2) eluted at 12 pore volumes as E2 and estrone (E1) in solution and sorbed on water-dispersible colloids (WDC).

	Conditions	$E2/E2_i$	$E1/E2_i$	E2 WDC/E2 WDC_i	Br^-/Br^-_i
		----------12 PV----------			
Clarion	Br^- and E2	0.01	0.03	-	0.95
	Br^-, E2, and soil WDC	0.78	0.15	0.12	0.95
	Br^-, E2 and swine manure WDC	0.40	0.16	0.08	0.97
Hanlon	Br^- and E2	0.11	0.90	-	0.99
	Br^-, E2, and soil WDC	0.30	0.14	*	1.03
	Br^-, E2 and swine manure WDC	-	0.01	-	1.01
Zook	Br^- and E2	0.08	0.13	-	1.01
	Br^-, E2, and soil WDC	0.23	0.12	0.11	1.02
	Br^-, E2 and swine manure WDC	0.21	0.16	0.09	1.00

E2 is the total E2 eluted in the aqueous phase, E1 is the total E1 eluted in the aqueous phase, E2 WDC is the total E2 eluted attached to colloids, $E2_i$ is the total aqueous E2 added, and E2 WDC_i is the total E2 attached to colloids added. Br^- is the total bromide eluted, and Br^-_i is the total bromide added. The * indicates that E2 on colloids was detected, but the mass fraction was too small to report in the table.

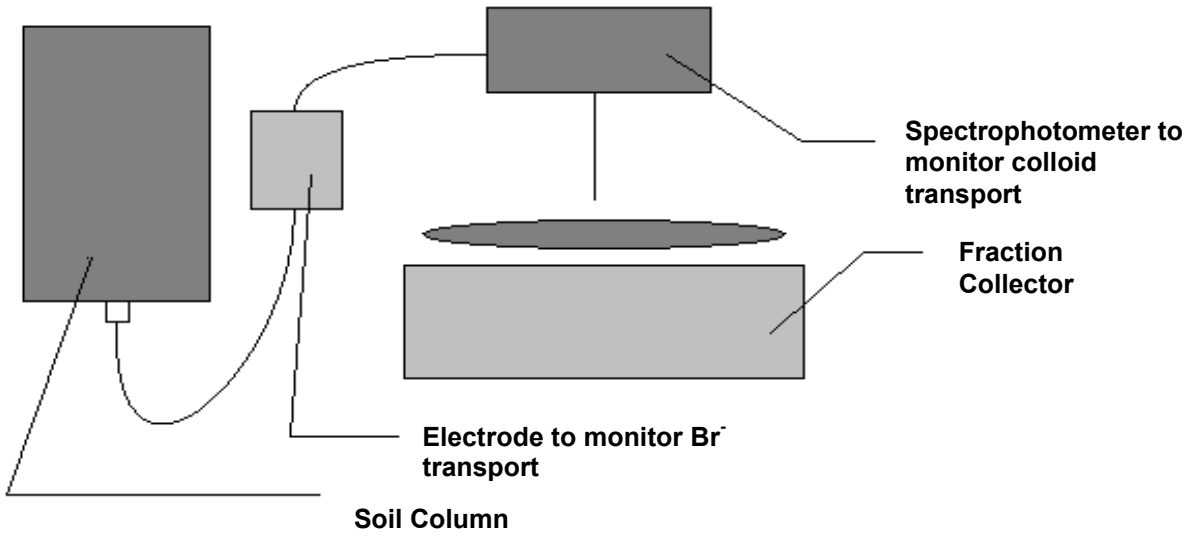


Figure 5-1. Column transport experimental set-up.

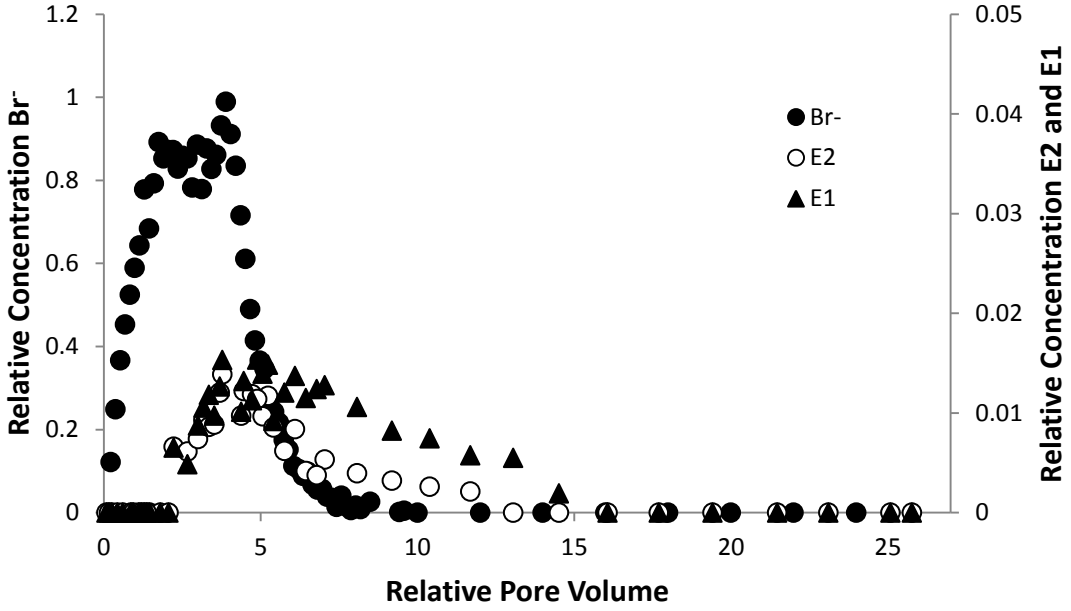


Figure 5-2. Clarion soil column breakthrough curve, bromide (Br^-), and 17 β-estradiol (E2) added including measurements of estrone (E1) in the effluent.

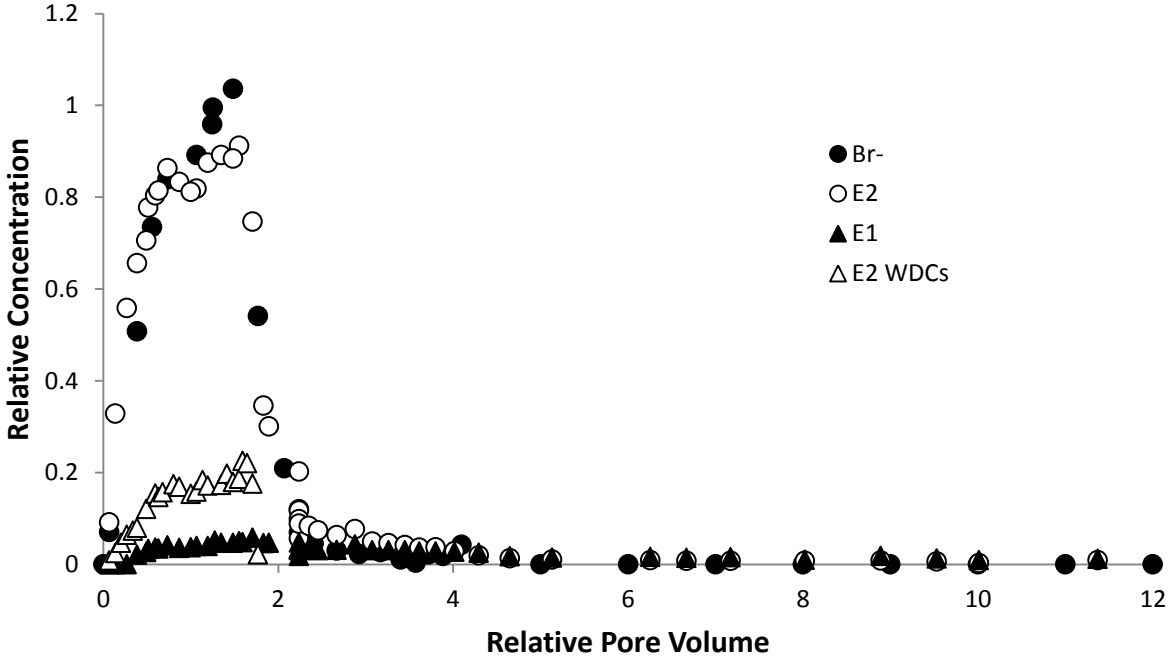


Figure 5-3. Clarion soil column breakthrough curve, soil water-dispersible colloids (WDCs) bromide (Br^-), and 17 β-estradiol (E2) added including measurements of estrone (E1) in the effluent.

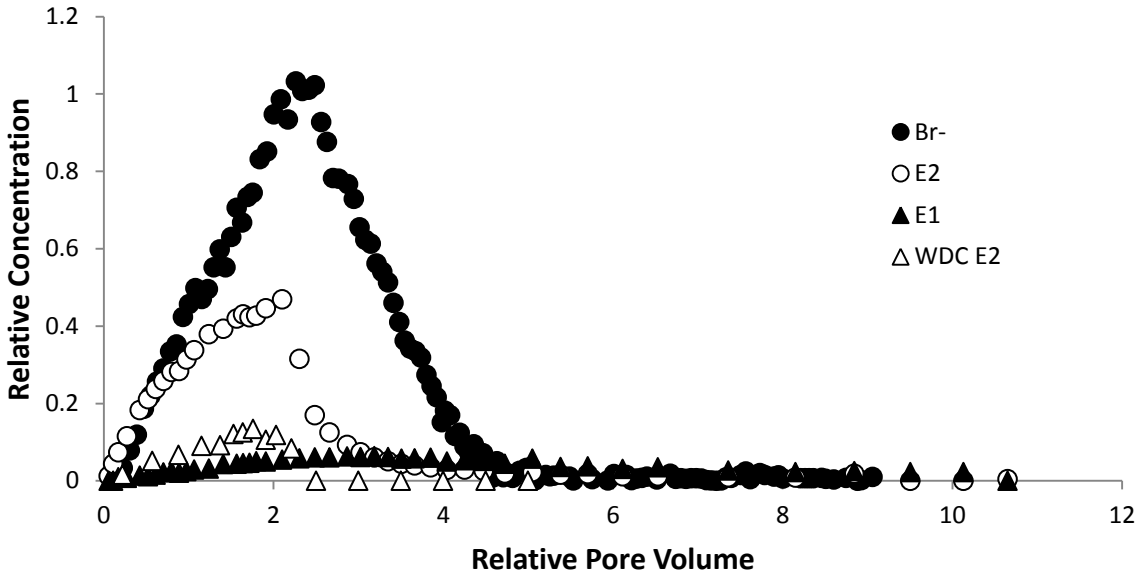


Figure 5-4. Clarion soil column breakthrough curve, swine manure water-dispersible colloids (WDCs) bromide (Br^-), and 17 β-estradiol (E2) added including measurements of estrone (E1) in the effluent.

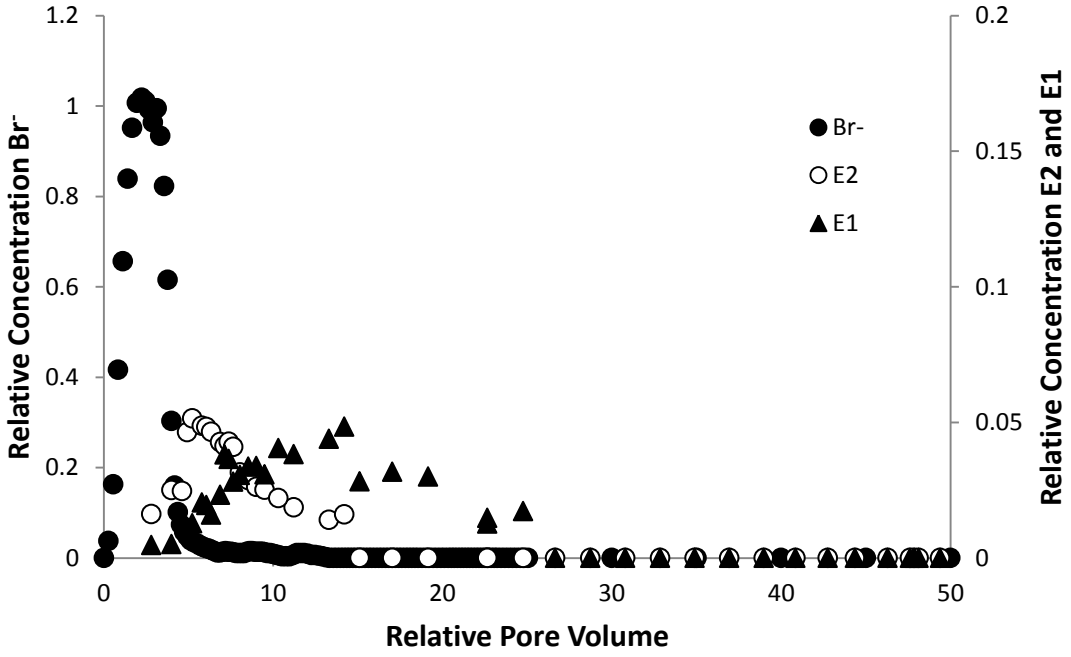


Figure 5-5. Hanlon soil column breakthrough curve, bromide (Br^-) and 17 β-estradiol (E2) added including measurements of estrone (E1) in the effluent.

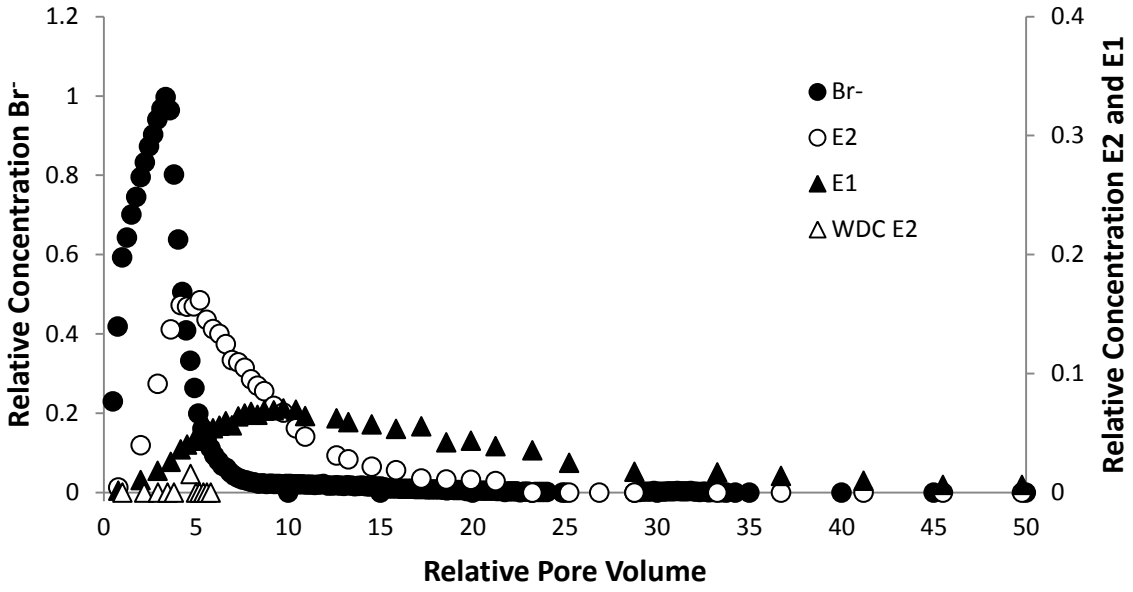


Figure 5-6. Hanlon soil column breakthrough curve, soil water-dispersible colloids (WDCs) bromide (Br^-), and 17 β-estradiol (E2) added including measurements of estrone (E1) in the effluent., and E2 added.

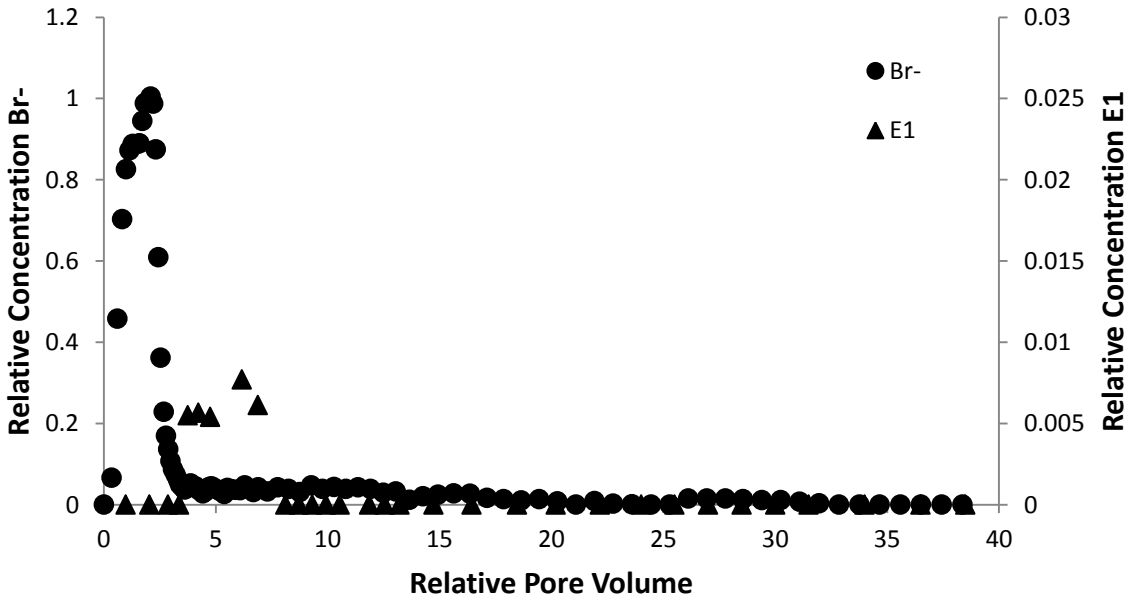


Figure 5-7. Hanlon soil column breakthrough curve, swine manure water-dispersible colloids (WDCs) bromide (Br^-) and 17 β-estradiol (E2) added including measurements of estrone (E1) in the effluent.

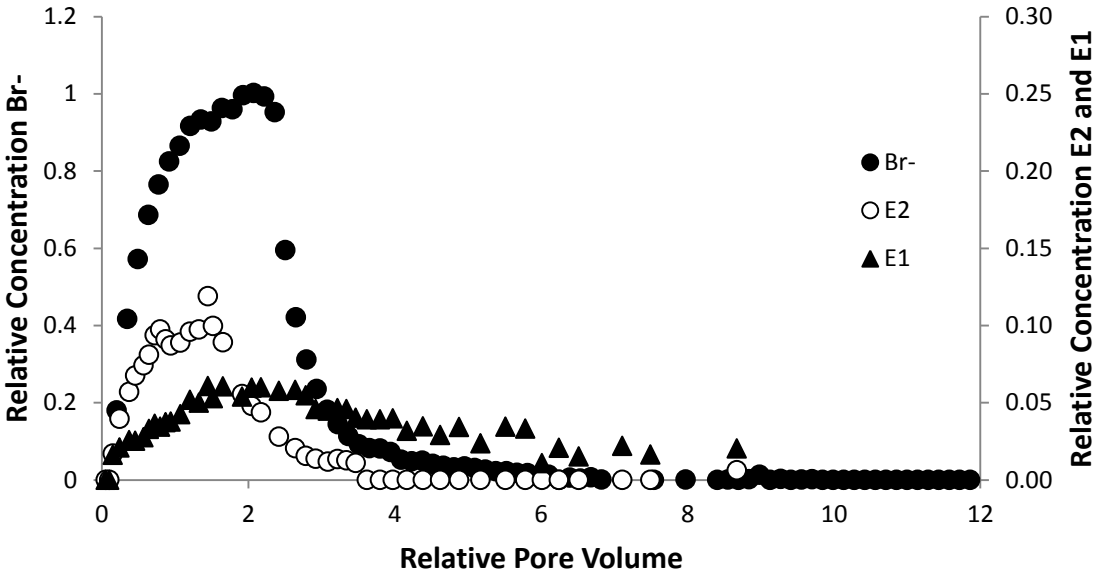


Figure 5-8. Zook soil column breakthrough curve, bromide (Br^-) and 17 β-estradiol (E2) added including measurements of estrone (E1) in the effluent.

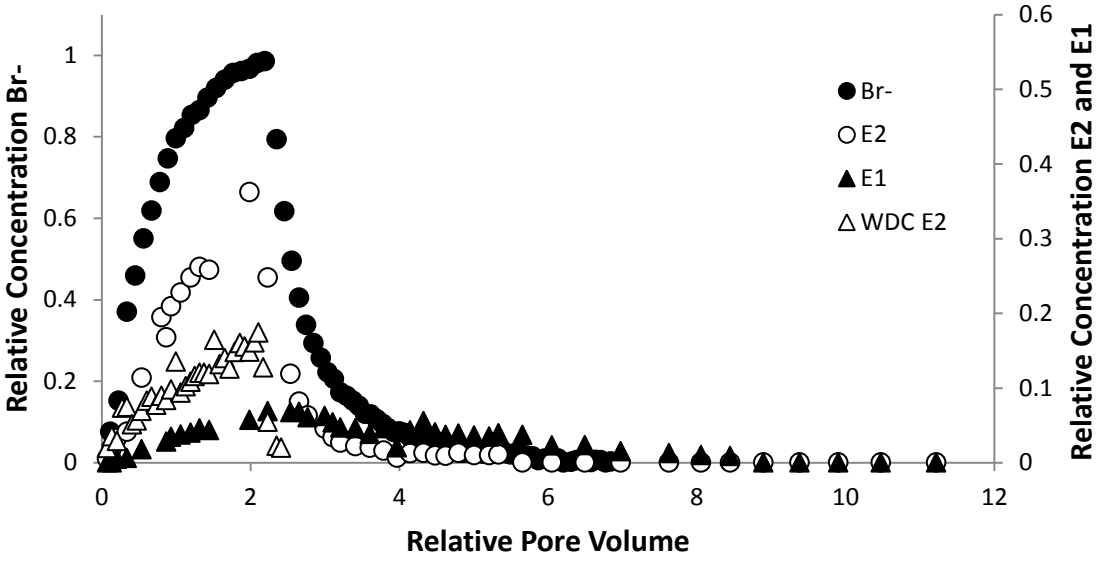


Figure 5-9. Zook soil column breakthrough curve, soil water-dispersible colloids (WDCs), bromide (Br^-), and 17 β-estradiol (E2) added including measurements of estrone (E1) in the effluent.

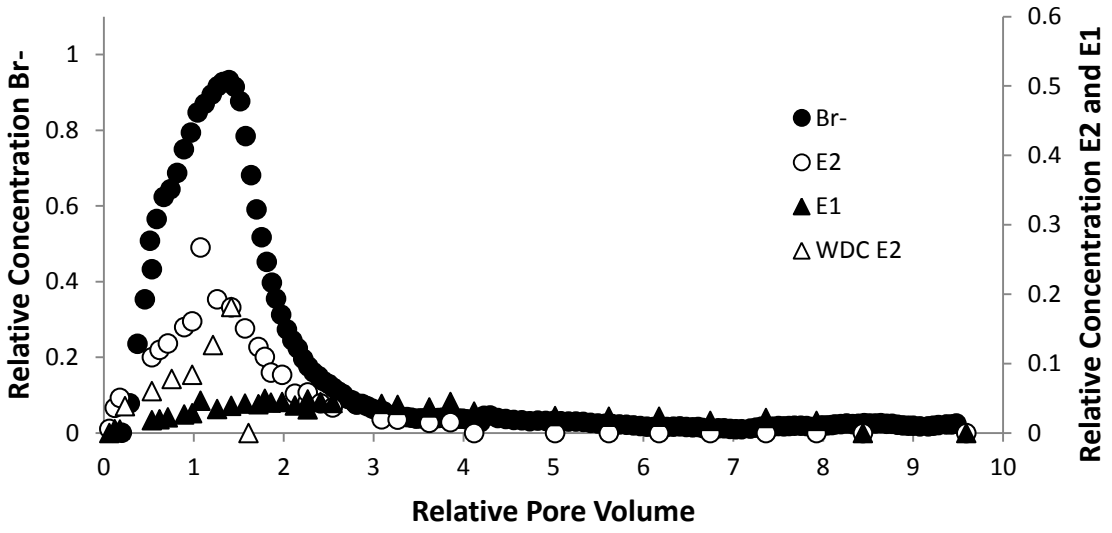


Figure 5-10. Zook soil column breakthrough curve, swine manure water-dispersible colloids (WDCs), bromide (Br^-), and 17 β-estradiol (E2) added including measurements of estrone (E1) in the effluent.

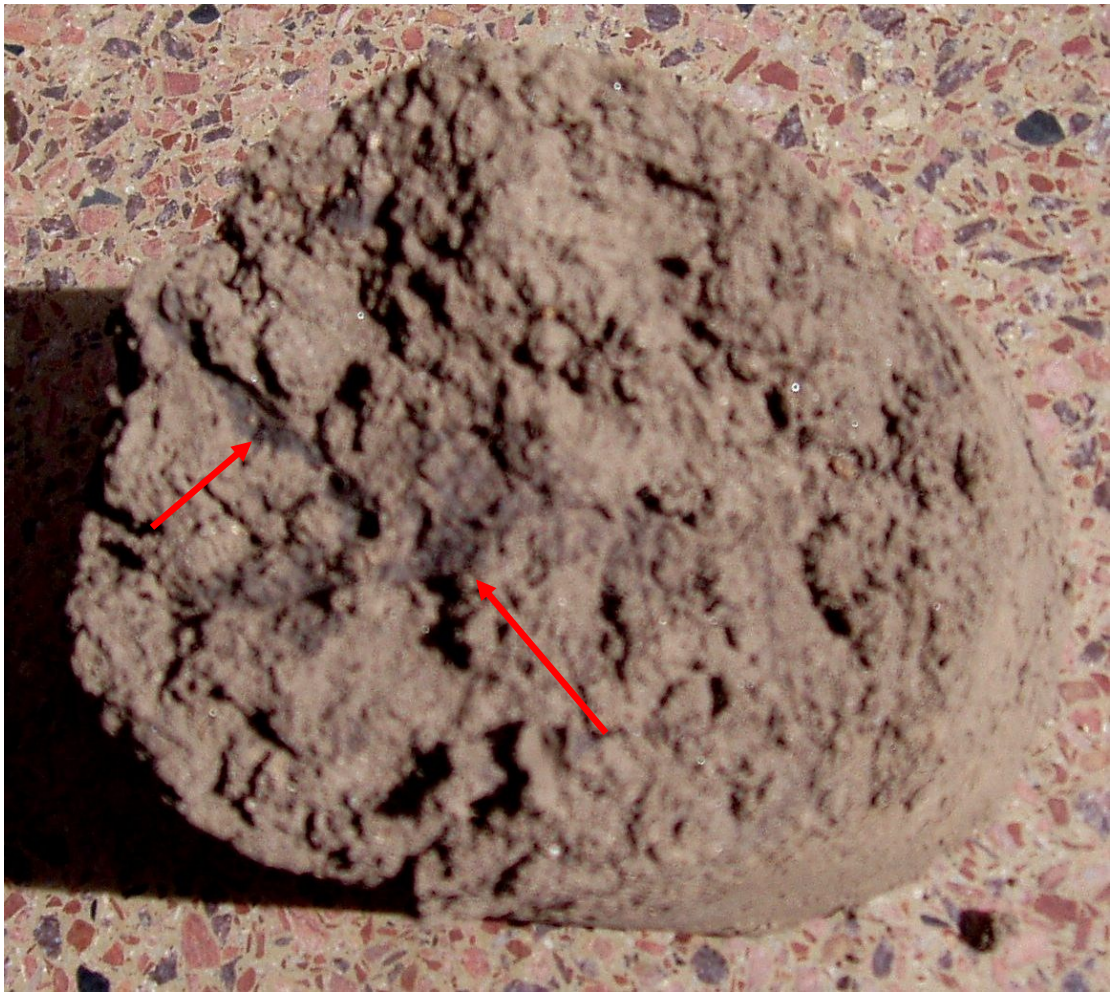


Figure 5-11. Clarion soil core with deposited soil colloids lining macropores. The darkened areas where colloids have been deposited along the interior of soil macropores are noted by red arrows.

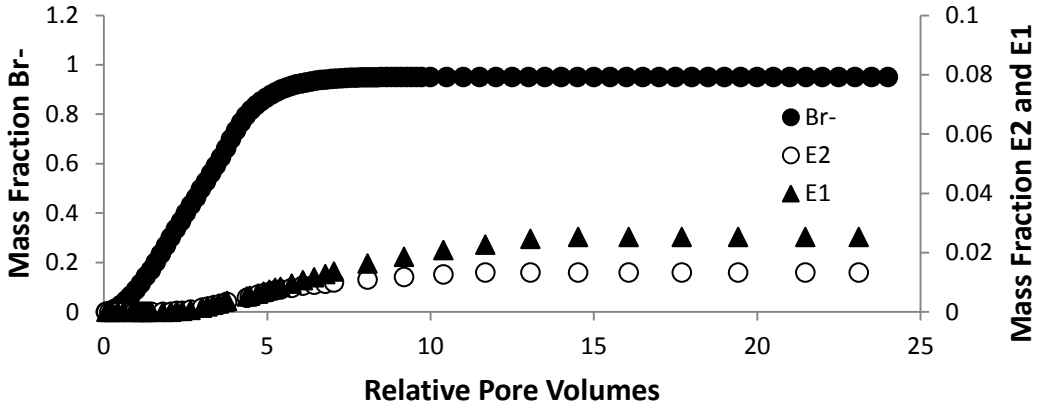


Figure 5-12. Mass fractions transported in Clarion with Br^- and E2 added mass fraction E2 transported.

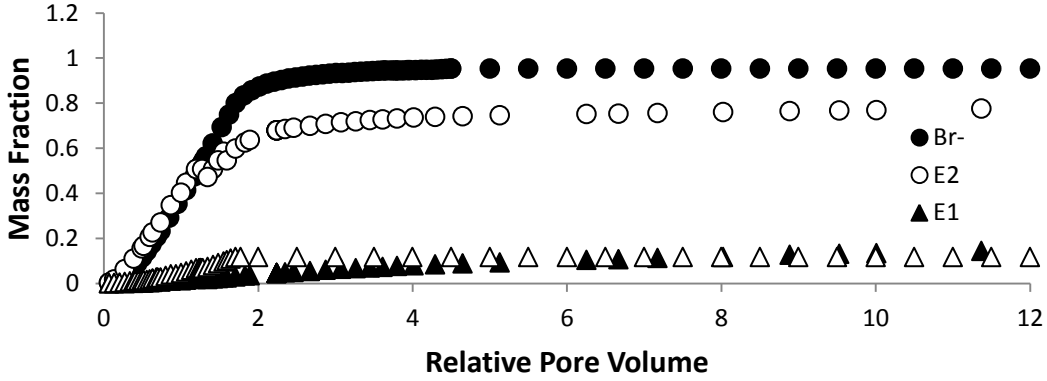


Figure 5-13. Mass fractions transported in Clarion with Br^-, E2, and soil water-dispersible colloids (WDCs) added mass fraction E2 transported.

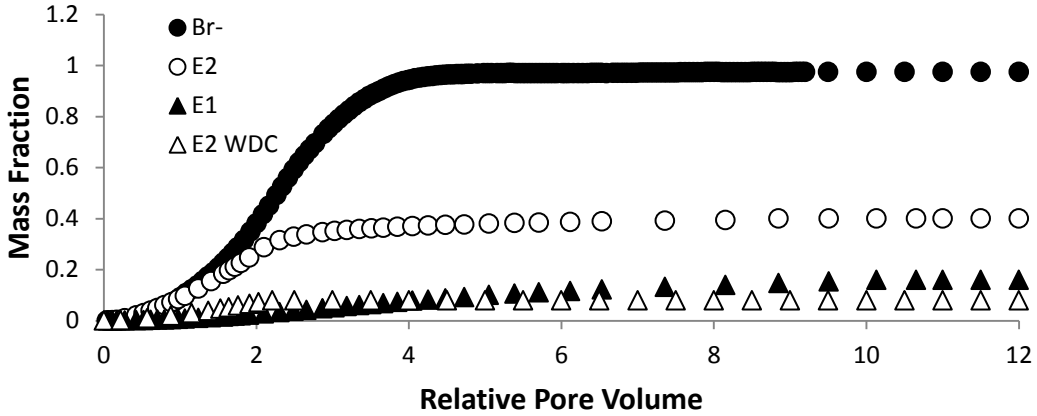


Figure 5-14. Mass fractions transported in Clarion with Br^-, E2, and swine manure water-dispersible colloids (WDCs) added mass fraction E2 transported.

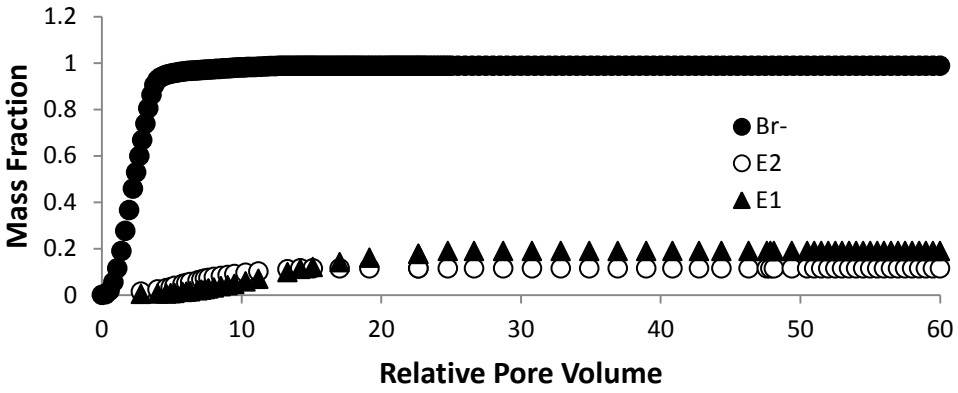


Figure 5-15. Mass fractions transported in Hanlon with Br^- and E2 added mass fraction E2 transported.

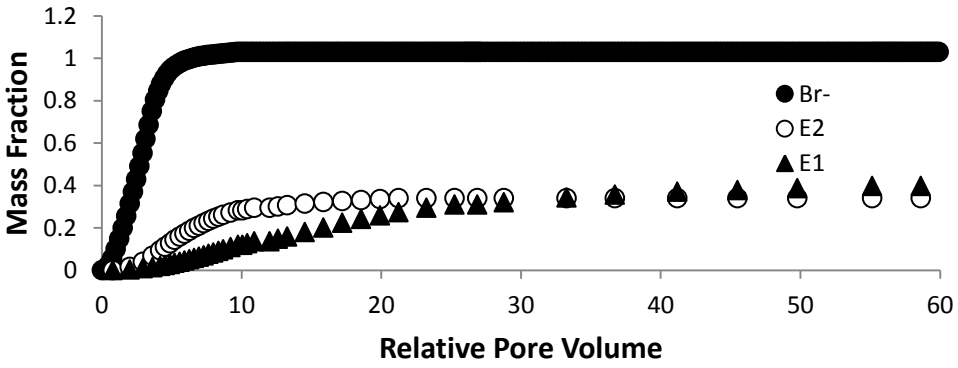


Figure 5-16. Mass fractions transported in Hanlon with Br^-, E2, and soil water-dispersible colloids (WDCs) added mass fraction E2 transported.

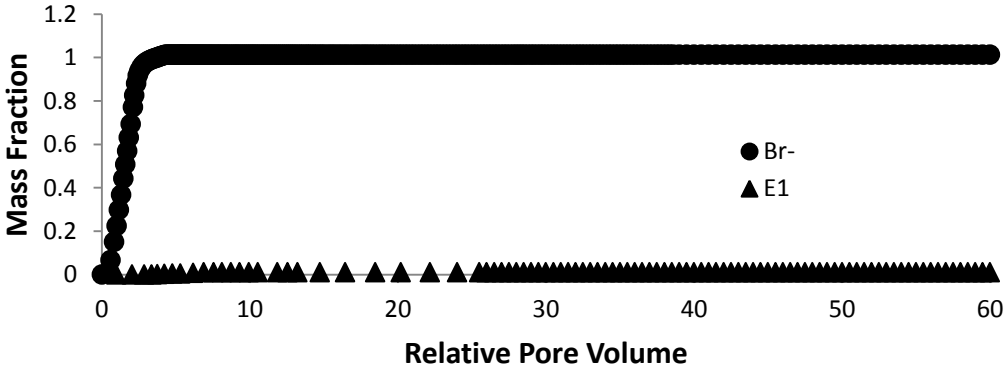


Figure 5-17. Mass fractions transported in Hanlon with Br^-, E2, and swine manure water-dispersible colloids (WDCs) added mass fraction E2 transported.

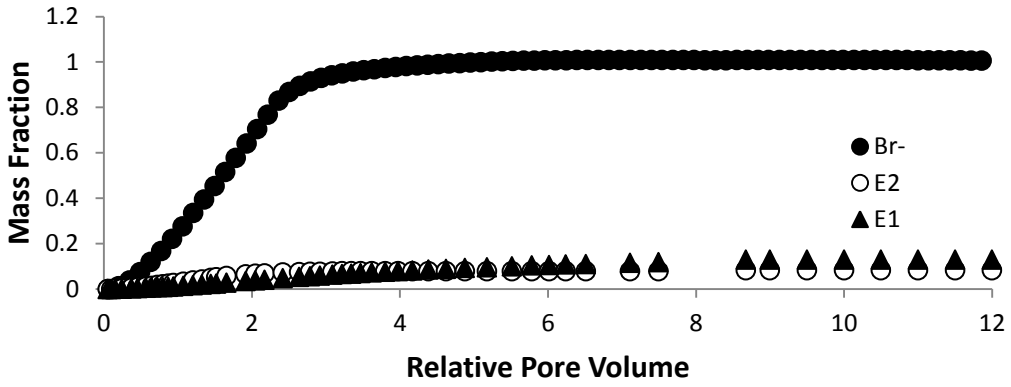


Figure 5-18. Mass fractions transported in Zook with Br^- and E2 added mass fraction E2 transported.

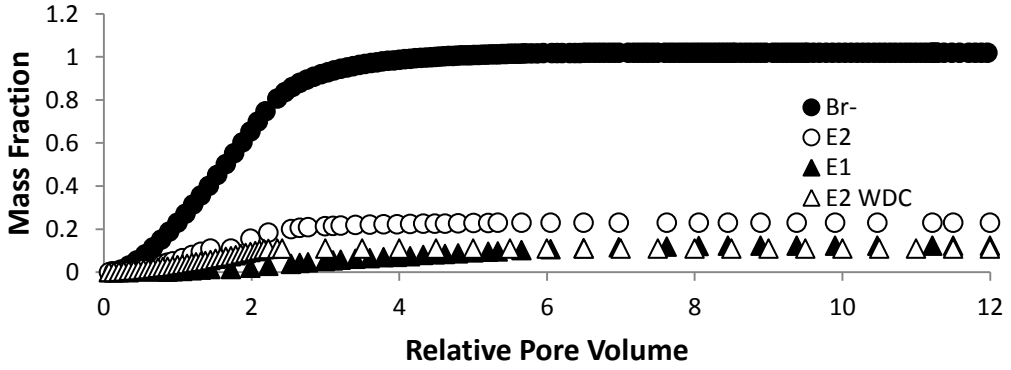


Figure 5-19. Mass fractions transported in Zook with Br^-, E2, and soil water-dispersible colloids (WDCs) added mass fraction E2 transported.

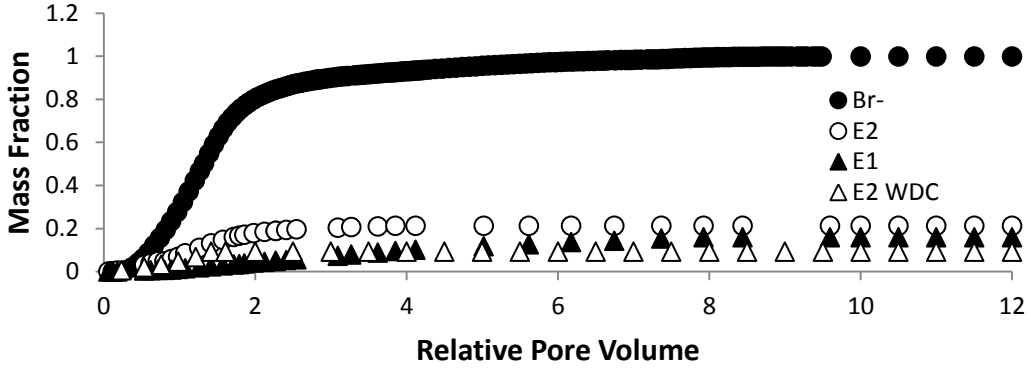


Figure 5-20. Mass fractions transported in Zook with Br^-, E2, and swine manure water-dispersible colloids (WDCs) added mass fraction E2 transported.

Chapter 6 : General conclusions and future research

General Conclusions

While estrogens in the environment continue to be of concern, our understanding of their pathways through soil which may impact surface water has been somewhat improved. From this work we may conclude that suspended colloids are a possible pathway of estrogen transport in soil. Conditions making this pathway more probable are: a large sorption potential for colloidal material, rapid sorption, slow sorbed-phase degradation, and the presence of preferential flow paths.

Estrogen sorption to soil and colloidal material is dominated by soil organic carbon as the sorbent, and organic carbon is present at higher concentrations in colloidal material (whether of soil origin or swine manure origin) than it is in whole soils. This means that, on a mass basis, colloids can sorb more estrogen than whole soil, and thus, that colloidal material has a large sorption potential for estrogens.

Estrogen sorption is best described as hydrophobic partitioning, and is controlled by solution concentrations at equilibrium. The kinetics of sorption show both a rapid phase and a second slower phase, with similar rates between the different materials measured. The proportion of the total sorption in each of these phases has some variability, but displays a trend towards greater sorption in the slow phase with increasing clay content. The impact of the rapid initial rate of sorption is that a large portion of estrogen can be removed from flowing pore water during transport, thus slowing the movement of estrogens and allowing increased degradation. Due to the presence of smectite in the mineral fractions of all the

materials (except the swine manure), and the similar pool sizes for each of these phases, we hypothesize that the two phases arise from the exterior and interior organomineral surfaces, and that the two rates derive from sorption and diffusion-limited sorption.

Degradation of estrogens is rapid in soil under aerobic conditions, but can slow, cease, or even reverse under anaerobic conditions. The environmental consequence is that estrogen persistence can increase if there is low oxygen, and potency may even increase under reducing conditions. Places where this may occur include: beneath swine manure lagoons, in fields where saturated conditions ensue shortly after manure application, in soils where preferential flow could transport colloids to shallow groundwater, and in wetlands that receive run-off from manure or other estrogen sources.

If preferential flow pathways are present, colloidal material can carry estrogens through soil. While colloids still moved in minute amounts through re-packed sand columns, preferential flow pathways were required for colloids to move rapidly and in large quantities. When present, colloids can facilitate the movement of soluble estrogens via a hitchhiking pathway, wherein estrogens are carried partway on colloids which then become entrapped and act as a source of desorbing estrogen. The presence of colloids and preferential flow paths during transport decreased the time of first detection, and increased both the concentration at peak delivery, and the total estrogen mass fraction transported. Colloids form a more rapid and effective path for estrogen transport if they are introduced to the soil with estrogens already attached, for example where there is run-off of an estrogen source already attached to colloidal solids, such as manure.

Future Directions

There are many possibilities to continue researching the fate and transport of E2 and E1 in soils, both from the measurements of this work, and by pursuing the implications of the results and conclusions of this work. Direct continuation of this research, based on these measurements, would include modeling of colloid-facilitated transport, and perhaps developing a model for the behaviors observed in the unsterilized batch studies, which may require some additional measurements. The implications of the results and conclusions of this work naturally raise many questions about the fate and transport of E2 and E1, specifically as they relate to microorganisms and oxidation/reduction potential of soils.

Modeling of the observed processes from this work could include the construction of models for both the static and dynamic (flowing) systems. The static system model would need to include several reactions and chemical species, as well as sorbed states and (potentially) different types of sorption sites. Additional measurements that might be needed to validate such a model would include both sorbed species and additional daughter products, allowing better estimation of mass balance. Modeling the flowing system is even more complicated. One interesting phenomenon in colloidal transport systems is that physical conditions may be changing with time. With relatively large concentrations of colloids in suspension, there is potential for surface pores to become clogged, and macropores to become lined with colloidal material. This clogging and pore lining represents a new area in soil physics research, with interesting connections to soil formation. A better understanding of this phenomenon could enable building a more comprehensive model of colloid-facilitated transport conditions, useful (for example) under high run-on erosion conditions.

Research into the fate and transport of E2 and E1 in the soil should also examine the role of microorganisms in the transformation of E2 to E1 and vice versa. Additionally, there should be investigation into the organisms and/or enzymes that may be involved in the degradation of E1, an irreversible reaction which represents the removal of nearly all estrogenic activity from an E2 source. Finally, transport experiments under reducing conditions might further our understanding of what may happen to E2 and E1 in wetlands, under swine manure lagoons, and in soils and geologic units (aquifers) with reducing conditions.

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